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EFFECT OF CLIMATE ON TIMBER-GROWTH FLUCTUATIONS[1]

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INTRODUCTION

The cross section of a forest tree tells its life story. Annual rings of medium width near the center reveal the tree's origin in an isolated position or an even-aged stand, for two-storied or many-aged stand conditions are shown by narrow ring widths. A series of wide rings usually follows, denoting the tree's vigorous prime, and narrowing rings near the circumference indicate its senescence. Within these broad age periods, cycles of narrower or wider rings suggest epochs of suppression by neighboring trees or dominance over them in the intense struggle for light and moisture; or they may imply periodic fluctuations of climate. Within such epochs, individual annual rings commonly show striking variation in width around the mean width for the epoch. Typical fluctuations of annual ring width are presented diagrammatically in figure 1.

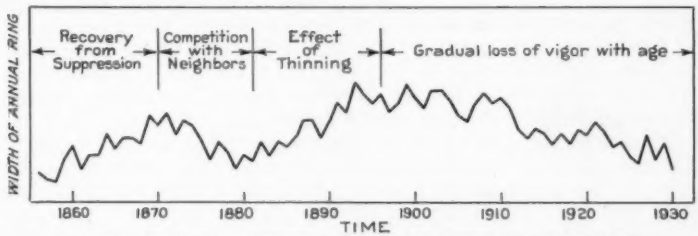

FIGURE 1.—Graphical representation of fluctuations in width of annual rings of a tree.

Insofar as competition with neighboring trees and other vegetation affects growth within an epoch, the magnitude of the growth of a given tree in an unthinned stand may be expected to be quite independent of that of other trees, chosen at random, during the same span of years; for two or more trees are probably not subject to the same hazards from this source during the same time intervals. All the trees of a stand, however, are exposed to the same general conditions of weather and periodic fluctuations of climate. Insofar as weather affects growth, therefore, the width of the annual rings of single trees, or the average width of those of groups of trees, should, in the same years, be highly correlated, particularly if the effects of the age cycle and of epochs of suppressive competition be eliminated.

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[2] During most of a year's leave of absence from Switzerland, Dr. Meyer acted as collaborator in the section of forest measurements of the Forest Service.

It has been generally recognized that variation in width of annual rings is associated with variation in climate (*1, 3, 4, 5, 8, 9, 10, 12, 13*).[3] So far as the present authors know, however, no attempt has been made to investigate the magnitude of the variation in annual growth that is due to fluctuation in climate. Such a measure is basic to judgment concerning the efficacy of a given silvicultural treatment in a regulated forest; for the comparison of periodic growth before and after treatment may be misleading unless the forester has some knowledge of the extent to which fluctuation in weather and climate may influence growth without change of treatment.

Meteorological conditions as a whole are among the most complex phenomena of nature, subject as they are to an infinite number of combinations, each of which may influence growth in a different way. While it would hardly be possible to explain their effects fully—that is, to find a functional relation between weather and growth—part of the effect of weather on growth may perhaps be explained, not as a functional relation but as a probable or conjectural relation, by the method of least squares. It is desirable to know, for instance, in what seasons and to what extent tree growth is affected by rainfall, by temperature, by bright sunshine, and so on. Positive answers to such questions should form valuable background for work in timber-tree physiology and ecology and in the practice of silviculture.

Meteorological phenomena cannot be dealt with in their entirety, but the effect of single factors on growth can be investigated. Usually any one such factor is correlated with others, which are, therefore, also dealt with, although indirectly; rainfall, for instance, is correlated with temperature and sunshine, and with relative humidity.

In this paper it is proposed (1) to divide the variation of annual growth of sample trees over a period of 63 years into that portion due to influence within and that to influence beyond the stand, to arrive at the magnitude of annual growth fluctuations that are due to outside influences, and to illustrate the resulting effects on changes in basal area and volume growth; and (2) to investigate in detail the effects of climate on timber growth, with special reference to rainfall distribution and its influence on annual-ring width.

TREE DATA

Dr. Meyer brought with him from Switzerland the tree data used, which were made available to him through Dr. Hermann Knuchel, professor of forestry at the Eidgenössische Technische Hochschule, Zurich. They consist of measurements of annual rings of 12 dominant white firs (*Abies alba* Mill.) taken from an even-aged stand near Aarau, Switzerland, for the years 1864–1926. Although the species and conditions may not be entirely comparable to any American species or environment, the methods used are equally appropriate to American timber types.

Concerning the trees and their environment, a translation of Knuchel's statement (*8, pp. 268–269*) is as follows:

> a large number of dominant sample trees had to be cut in an approximately 120-year-old fir-spruce-beech stand of the Swiss central highlands.
>
> The stand in question forms part of the so-called "lower forest" of the Zofingen Commune, 480 meters above sea level on a gently inclined northern slope of a Tertiary sandstone hill. The marly-loam soil is principally of glacial origin. The

[3] Reference is made by number (italic) to Literature Cited, p. 107.

average annual precipitation based on a 50-year record is about 1,050 mm; the average annual temperature is 8.3° C.

The stand had been only slightly thinned up to a few years prior to the removal of the sample stems; at intervals of 5 and 6 years suppressed and dying trees only had been removed. In the last 20 years the thinnings were somewhat heavier, but the stand in 1926 was still fairly dense. The rate of growth of the spruce had fallen off somewhat, whereas the firs were still in full development. This fact induced us for the present to use only *Abies pectinata* D. C. [*Abies alba* Mill.] in the investigation of increment fluctuations.

Of the 12 sample stems cross sections were taken at ground level and at heights of 4, 8, and 12 m. The height of the trees was 32 to 38 m; the diameter breast high was 54 to 68 cm. The central rings showed that the firs had originated naturally and that cutting of the old stand had taken place when the firs were 10 to 30 years old and 1 to 6 m high. On the basis of the annual rings we are justified in assuming that the trees had subsequently always been dominant.

Width of individual rings at each crosscut were measured along three radii removed from each other by about 120°, and the average of these for each year entered as the tree-year observation. The present data are the measurements taken on the crosscuts 4 m above the ground. These are presented graphically in figure 2. Even in this raw form, correlation between trees in the different years is apparent. Deviations of annual ring widths from trends of the 12 individual trees for the 63-year period are given in table 1, the last column of which lists all the variation that is due to meteorological conditions from year to year plus an element due to random error.

The rainfall data are the monthly precipitation records at Aarau, Switzerland, from June 1863 to August 1926 (*14*).

TABLE 1.—*Deviations of annual ring width from trends of individual trees, in units of 0.01 mm*

Year	Deviations of tree no.—												Sum	Mean
	1	2	3	4	5	6	7	8	9	10	11	12		
1864	45	50	17	8	−4	8	8	24	−17	47	48	44	278	23
1865	−48	−39	−38	−20	−70	−90	−20	−84	−26	−12	−72	−46	−565	−47
1866	7	30	−15	22	11	−17	22	9	54	39	18	25	205	17
1867	12	29	38	54	3	26	54	11	27	40	38	56	388	32
1868	−14	−42	2	25	−35	−31	25	−27	8	−9	−23	−13	−134	−11
1869	−19	−22	16	−23	−22	−9	−23	5	19	−28	−13	−23	−172	−14
1870	−15	−43	29	29	−70	4	29	28	20	2	−3	−12	−2	0
1871	40	36	12	20	2	−3	20	10	32	23	17	28	237	20
1872	−16	−45	6	2	−36	−20	2	−38	−37	14	−3	9	−162	−14
1873	48	14	29	42	27	52	42	64	4	5	26	29	382	32
1874	12	14	32	5	−1	45	5	36	55	6	−34	10	185	15
1875	6	−47	−5	−33	1	−12	−33	−41	−23	−14	−14	−10	−225	−19
1876	0	−28	18	−12	−6	0	−12	−19	−2	17	−64	−20	−128	−11
1877	−17	−9	−39	−30	16	3	−30	3	9	−12	−34	10	−130	−11
1878	−23	10	−36	−9	−2	16	−9	15	30	9	15	10	26	2
1879	−10	−20	−13	3	0	39	3	−63	22	−10	25	0	−24	−2
1880	−27	−21	−61	−56	−17	−39	−56	−50	−37	0	−5	−30	−399	−33
1881	7	−12	−18	−4	45	34	−4	42	14	1	15	−20	100	8
1882	50	37	45	−3	27	67	−3	44	35	32	75	30	436	36
1883	43	16	37	8	19	70	8	46	7	13	54	29	350	29
1884	45	16	30	0	12	22	0	29	−22	−16	24	19	159	13
1885	47	5	2	1	4	35	1	21	−51	−6	24	−1	82	7
1886	−9	−6	14	−70	−34	18	−70	−27	−70	−45	4	−22	−317	−26
1887	24	−57	−24	−27	−32	−58	−27	−5	−109	6	−26	−32	−367	−31
1888	−34	−67	−62	−66	−39	−57	−66	−33	−77	−53	−47	−63	−664	−55
1889	−32	2	1	−24	13	−24	−24	−10	−36	−12	23	−14	−137	−11
1890	−19	31	−27	7	15	−11	7	22	−15	−2	3	−14	−3	0
1891	−17	20	−26	−12	−13	11	−12	−16	−34	−31	13	−25	−142	−12
1892	−25	−1	−24	−1	0	−16	−1	−14	−42	−30	−27	−26	−207	−17
1893	−23	−31	−42	10	12	−3	10	−42	−1	−19	−8	−27	−164	−14
1894	−31	−2	−20	−29	14	−10	−29	1	−10	−8	12	−28	−140	−12
1895	−30	−33	−39	−58	−44	−48	−58	−37	−89	−28	−58	−39	−561	−47
1896	−18	−24	−17	−7	9	−35	−7	−25	−17	−7	2	−40	−186	−15
1897	−36	5	−36	−16	−9	−62	−16	−13	−46	−36	−39	−32	−336	−28
1898	−25	45	−34	4	−7	−20	4	0	15	−5	1	−53	−75	−6
1899	6	24	−13	−15	46	−7	−15	2	−4	−4	−9	−4	7	1
1900	−22	43	−12	6	28	6	6	−6	−3	−14	−29	−15	−12	−1

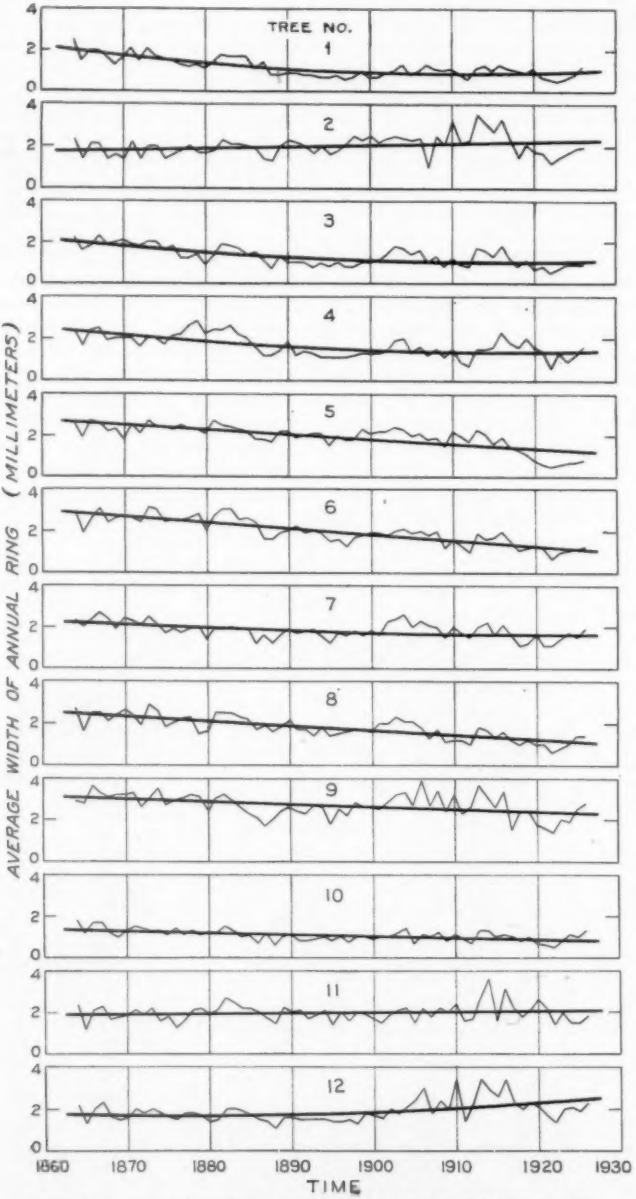

FIGURE 2.—Annual ring widths and tree trends of 12 dominant fir trees, 1864–1926.

TABLE 1.—*Deviations of annual ring width from trends of individual trees, in units of 0.01 mm*—Continued

Year	Deviations of tree no.—												Sum	Mean
	1	2	3	4	5	6	7	8	9	10	11	12		
1901	−21	12	−1	−13	40	9	−13	35	−11	−3	−49	−27	−41	−3
1902	0	31	30	58	42	2	58	38	30	−2	−10	11	288	24
1903	10	41	71	68	65	24	68	70	61	19	10	−10	497	41
1904	41	30	62	89	57	37	89	53	72	40	20	18	608	51
1905	−8	19	33	30	29	20	30	55	14	−30	−50	46	188	16
1906	12	28	54	60	41	32	60	27	135	11	20	104	584	49
1907	43	−112	−5	41	14	15	41	−21	16	2	−21	−18	−5	0
1908	23	37	25	31	26	38	31	22	87	23	19	40	402	33
1909	14	−4	−24	−18	−12	−39	−18	−36	−11	−16	−1	−2	−167	−14
1910	24	105	16	32	60	3	32	−24	70	14	39	136	507	42
1911	−6	4	−13	3	43	−24	3	−22	−19	−5	−41	−66	−143	−12
1912	−26	14	−23	−17	15	−51	−17	−40	12	−24	−32	22	−167	−14
1913	23	133	68	34	77	42	34	43	123	37	68	130	812	68
1914	43	92	58	54	59	14	54	35	65	38	158	77	747	62
1915	13	51	28	−6	12	27	−6	−3	16	8	−42	45	143	12
1916	42	110	78	45	44	60	45	29	87	19	108	122	789	66
1917	22	10	18	−5	6	13	−5	−8	−92	10	17	10	−4	0
1918	11	−71	−22	−55	−11	−25	−55	−26	0	−9	−23	−23	−309	−26
1919	0	−12	8	−44	−29	−12	−44	−4	1	12	7	4	−113	−9
1920	19	−43	−33	−4	−57	0	−4	−22	−58	−18	57	−18	−181	−15
1921	−22	−54	−23	−54	−75	−7	−54	−20	−77	−27	17	−61	−457	−38
1922	−33	−104	−53	−54	−82	−54	−54	−57	−95	−36	−64	−94	−780	−65
1923	−44	−75	−34	−24	−70	−21	−24	−35	−34	−5	−4	−37	−407	−34
1924	−36	−56	−14	6	−58	−8	6	−13	−43	26	−54	−30	−274	−23
1925	−17	−37	−15	−14	−56	4	−14	29	18	16	−54	−53	−193	−16
1926	21	−27	−16	26	−43	17	26	32	50	47	−24	−16	93	8

MAGNITUDE OF ANNUAL GROWTH FLUCTUATIONS

ANALYSES OF CURRENT ANNUAL GROWTH

Dominant trees in an even-aged stand are free—and have probably been free throughout most of their earlier life—from epochs of severe suppression by neighboring trees. As a consequence, progressive changes in their growth rates with respect to age (as distinct from changes with respect to time) should be subject to elimination by fitting trend curves, of first or second degree only, to the data of each tree. These should be sufficient to eliminate the effects of age on growth without danger of eliminating important changes in increment due to climatic influence.[4] A straight line was therefore fitted to the values of trees nos. 2, 5, 6, 8, 9, 10, and 11, and a parabola to trees nos. 1, 3, 4, 7, and 12 (fig. 2).

The residuals from these trends (table 1) were made the basis for a number of preliminary comparisons between the trees. Correlation coefficients between the annual growths of paired trees are as follows:

	Correlation coefficient
Trees 1 and 2	0.422
Trees 1 and 3	.633
Trees 3 and 4	.510
Trees 4 and 7	.310
Trees 5 and 6	.512
Trees 7 and 8	.586
Trees 9 and 10	.594
Trees 11 and 12	.608

On the hypothesis that they express the relationship found between samples of independent variates, these correlation coefficients would

[4] This statement is based on the assumption that if the 63-year climatic record can be expressed as a function of time, the function is more complex than would be expressed by a curve of first or second degree only.

be distributed around zero with standard deviation of about 0.077. In the face of an average correlation coefficient above 0.5, however, such a hypothesis is untenable. On the other hand, with these moderate coefficients of correlation, there is a great deal of variation in annual growth between single trees unexplained by a system of causes outside the stand. If this unexplained variation is peculiar to the individual trees or to random error in measurement of the annual rings, it is subject to considerable reduction if the residuals from trend in each year for the 12 trees are averaged.

As a step in this direction, correlation coefficients were calculated between average residuals of two sets of six trees each:

	Correlation coefficient
Trees 1, 2, 3, 4, 5, 6, and 7, 8, 9, 10, 11, 12	0.920
Trees 1, 3, 5, 7, 9, 11, and 2, 4, 6, 8, 10, 12	.945

These high values demonstrate clearly that factors external to the stand have accounted for the bulk of the variation between the averages of annual growths, based on as few as six trees each. The probability that these factors are anything other than weather or climate seems extremely remote.

The best estimate of the magnitude of variation in ring width that is due to fluctuations of climate will be arrived at from an analysis of variance (*15*) of the raw data of figure 2. From the classification in table 2, it is evident that a large proportion of the sum of squares of the deviations of each tree-year observation from the general mean of the annual ring widths of the 756 (12 times 63) observations is due to differences between trees. Subtracting this quantity from the total, the remaining sum of squares is within the trees, and this in turn is divided into sums of squares (table 3) that are eliminated by the trends (age progression), by differences in years (effect of weather and climate), and by the residual, this last to be taken as the basis for determining random variation. Mean squares for these quantities are given in the last column of table 3.

If the variation in the annual growth of these trees from year to year, after eliminating the effects peculiar to the individualities of the trees themselves and to changes in their ages, were entirely fortuitous, the mean square due to years would be expected to be practically identical with the mean square in residual variation; in fact, from the distribution of F as given by Snedecor (*15, pp. 88–91*) a ratio of the former to the latter higher than 1.25:1, when based upon the same number of degrees of freedom as in this case, is to be expected only about once in every 100 random samples. The observed ratio of about 15:1 is therefore taken as proof that the variation in growth from year to year is not the outcome of a constant system of chance causes. Again, it is seen to be due to factors external to the stand, and these can hardly be anything other than changes in weather and climate.

TABLE 2.—*Division of the sum of squares into portions between and within trees*

Source of variation	Sum of squares	Degrees of freedom
Between trees	1,328,800	11
Within trees	1,764,480	744
Total	3,093,280	755

TABLE 3.—*Division of the sum of squares within trees into portions due to trends, years, and remaining variation; and pertinent mean squares*

Source of variation	Sum of squares	Degrees of freedom	Mean square
Tree trends	602,336	[1] 17	40,726
Years	620,694	62	10,011
Residual variation	451,450	665	679
Total	1,764,480	744	

[1] The 7 trends represented by straight lines account for 7 degrees of freedom; the 5 trends represented by parabolas account for 5×2=10 degrees of freedom; the sum of these is 17.

The mean square between years is 12 times the variance of the 63 annual ring widths (as measured from the tree trends) of the average tree. It contains one portion of variance due to the residual variation (*7, pp. 293–296*). The variance of annual ring width due to fluctuations of climate is therefore

$$\frac{10{,}011-679}{12}=777,$$

and its standard deviation is the square root of this quantity, or 27.8, in hundredths of millimeters. This is the magnitude sought. It may be taken as characteristic of the forest represented by these trees, because the growth of the entire forest is measured primarily in the growth of its dominant trees.

Had the residual mean square of table 3 been zero, the variance between years would have been

$$\frac{10{,}011}{12}=834,$$

and it would have been concluded that, after eliminating the differences between trees and their age changes, fluctuations of climate account for the whole of the remaining variance. Actually, fluctuations in climate account for $\frac{777}{834}$, or 93 percent, of the remaining variance—a truly noteworthy contribution of weather and climate to timber growth.

The general mean of the annual ring widths during the 63-year period is 177.9 in hundredths of millimeters. The standard deviation due to annual fluctuations in climate is therefore 15.4 percent of this mean. In 1 out of 20 years, accordingly, fluctuations in climate may be expected to be great enough to result in departures in diameter growth of more than 30 percent of the longer time average. If the variation in climate from year to year is itself the result of a multiplicity of causes no one of which predominated, variation in growth in periods of k years would be $\frac{15.4}{\sqrt{k}}$ percent. The relative magnitude of periodic growth fluctuations of the stand represented by these trees which must be considered to be due to changes in climate is shown in table 4.

TABLE 4.—*Coefficient of variation of periodic diameter growth*

Length of period (years)	Deviation from 63-year mean		Length of period (years)	Deviation from 63-year mean	
	Standard deviation	Three times standard deviation		Standard deviation	Three times standard deviation
	Percent	*Percent*		*Percent*	*Percent*
1	15.4	46	15	4.1	12
5	6.9	21	20	3.4	10
10	4.9	15			

FLUCTUATIONS IN CURRENT ANNUAL GROWTH IN BASAL AREA AND VOLUME

The effect of fluctuations of climate on basal area or volume increment may be deduced from two expressions: (1) The expression of basal area or volume in terms of diameter, and (2) the effect of fluctuations of climate on diameter increment.

For the expression of both basal area and volume in terms of diameter we may take

$$v=cd^a$$

in which d is tree diameter, c is a constant, and v is basal area if $a=2$, or volume if a is between 2 and 3. The value of a for volume determination depends upon definition of volume, species, etc.

If the annual diameter increment of a tree be denoted by i, and the standard deviation in i that is due to fluctuations in climate, expressed as a ratio of i, be denoted by p (the value of $100p$ for these data being 15.4 as determined above), then i is subject to an error pi on account of fluctuations in climate. These definitions lead to the following determinations:

(1) Without considering the fluctuations of climate:

	Diameter	*Basal area or volume*
Beginning of period	d	cd^a
End of period	$d+i$	$c(d+i)^a$
Increment during period	i	$c(d+i)^a-cd^a$

(2) Considering the fluctuations of climate:

	Diameter	*Basal area or volume*
Beginning of period	d	cd^a
End of period	$d+i\pm pi$	$c(d+i\pm pi)^a$
Increment during period	$i\pm pi$	$c(d+i\pm pi)^a-cd^a$
Increment fluctuation	$\pm pi$	$c(d+i\pm pi)^a-c(d+i)^a$
Increment fluctuation in percent of increment	$\pm 100p$	$100\left[\frac{(d+i\pm pi)^a-(d+i)^a}{(d+i)^a-d^a}\right]$

Expanding the expression of increment fluctuation in percent of increment for basal area and volume by the binomial theorem, and retaining the first two terms only, in both numerator and denominator, we obtain

$$100\left[\frac{(d+i)^a+a(d+i)^{a-1}.\quad(\pm pi)+\text{----------}-(d+i)^a}{d^a+ad^{a-1}.\quad i+\text{----------}\quad-d^a}\right]=$$

$$100\left[\frac{a(d+i)^{a-1}.\quad(\pm pi)}{ad^{a-1}.\quad i}\right]$$

and this reduces to

$$100\left[1+\frac{i}{d}\right]^{a-1} \cdot (\pm p) \text{ approximately.} \qquad (1)$$

If, for example, the average tree is 30 cm in diameter, and its relative increment $\frac{i}{d}$ is 0.10 in 10 years, the fluctuation to be expected in basal area increment in the next 10 years as a result of the caprice of climate is

$$\pm 100p(1+0.10)^{2-1}=\pm 100p(1.10),$$

that is, the expected relative fluctuation in basal area increment is 10 percent greater than that of diameter increment. The expected relative fluctuation in volume increment, giving a the value 2.5 (*2, p. 279*) would be $\pm 100p(1+0.10)^{2.5-1}=\pm 100p$ (1.15), or about 15 percent greater than that of diameter increment.

It is at once apparent that the relative fluctuation in both basal area and volume with respect to that of diameter depends upon the ratio $\frac{i}{d}$; that is, it varies directly with diameter increment of each tree—which, in turn, depends upon the annual growth of the tree and the length of period chosen—and inversely with the diameter of the tree itself. Hence, while real and positive, it is hardly sufficiently great to warrant its calculation for every tree, unless small timber of fast growth is being dealt with and longer periods than customary are used. The application of the relative fluctuation of the tree of average diameter (by basal area or by volume as the case may be) to the periodic growth of all the trees is sufficient approximation.

ELIMINATION OF CLIMATIC FLUCTUATIONS IN MAKING COMPARISONS OF PERIODIC INCREMENT

When periodic increments of a forest or sample plot have been determined from periodic inventories, comparisons between them are usually made for the purpose of estimating the effect on growth of a treatment, epidemic, or catastrophe, such as thinning, insects, or fire. The foregoing analyses, however, demonstrate that periodic changes in climate affect increment to a marked extent, independent of treatment or hazard. It may be necessary, therefore, to eliminate so far as possible the effect of climatic change in order to arrive at a valid estimate of the effect of treatment or other action.

In the simple case of three inventories, or two periodic increments, it is not necessary to specify any particular method of increment calculation. Obviously the more accurate the measurements and the method, the more reliable the results. Only the adjustment of the periodic increments to the values to be expected if the effects of climate were the same during the two periods need be considered.

The data upon which adjustment of this nature is to be based should be measurements of annual ring widths of 10 to 20 dominant trees of average size during a number of years equivalent to several periods during which the trees were relatively free from epochs of suppression by neighboring trees and from the influence of the treatment, epidemic, or catastrophe which is under investigation. If such trees cannot be spared from the sample plots or forest where periodic increments are

to be compared, they should, at least, be taken on the same species, same site quality, and in the immediate vicinity. Crosscuts along which measurements are made should, obviously, be above the influence of butt swell.

The data may then be subjected to analyses as discussed above. The effect of age may be eliminated by plotting the annual ring widths of each tree on time, and fitting a straight line or simple curve by freehand methods. It is not necessary to use the method of least squares, although with straight-line trends the work involved would not be great.

Deviations from the trends may be classified as in table 1, according to tree and year, and the average (algebraic) deviation of the trees for each year computed. These may then be plotted on a graph as deviations from a base horizontal line representing the general mean annual ring width of all measurements. If the graduations of the vertical scale are doubled, the results, as shown in figure 3, express the average

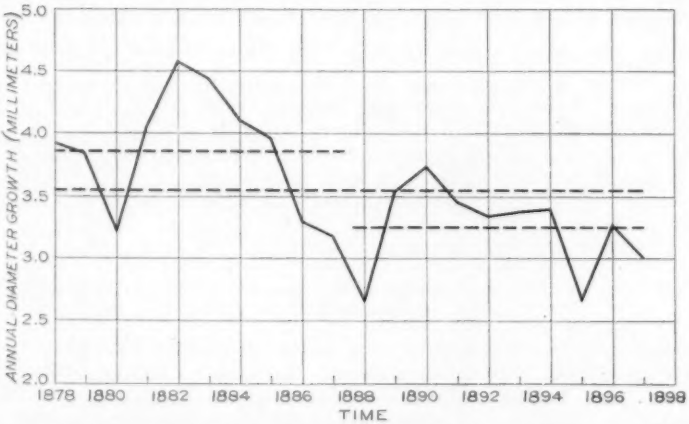

FIGURE 3.—Current annual diameter growth, corrected for age; periodic annual average for each of two periods, and general average diameter growth.

of the annual diameter increments, independent of the individual trends. The graphs of figure 3 show:

	Mm
Average diameter growth, first period	3.85
Average diameter growth, second period	3.25
General mean	3.55

Considering the diameter-growth fluctuation (due to climate) of the mean tree of the forest or sample plot as running parallel to that of these sample trees, it may be adjusted by the correction factors:

$$\frac{3.55}{3.85}=0.922 \text{ for the first period, and}$$

$$\frac{3.55}{3.25}=1.092 \text{ for the second period.}$$

In order to make adjustment in basal area or volume increment, the diameter of the tree of average basal area or volume is first calculated. If the diameter of the tree of average basal area is 30.0 cm, or 300 mm, to a satisfactory degree of approximation, $\frac{i}{d}$ of equation (1) for a 10-year period may be taken as

$$\frac{i}{d}=10\cdot\frac{3.55}{300}=0.118$$

Now we have first to calculate the factor p, i. e., the deviation of i expressed as a ratio of mean i; that is,

$$p=\frac{\pm 0.30}{3.55}=\pm 0.085.$$

The expected growth fluctuation then in basal area is, according to equation (1)

$$100\ (\pm 0.085)(1+0.118)^{2-1}=\pm 9.5 \text{ percent.}$$

The basal area increment as obtained for the first period should therefore be reduced by 9.5 percent and the basal area as obtained for the second period should be increased by 9.5 percent in order to eliminate the influence of differences in climate in the two periods.

INFLUENCE OF PRECIPITATION

METHOD OF STUDY

The methods used by Fisher (*6*) in studying the influence of rainfall on the yield of wheat in England formed the basis for the methods used in the present investigation.

The problem is the calculation of the average influence of the rainfall distribution during a period of several seasons, upon the width of the current annual ring of dominant trees. The length of the period should obviously be at least that of the growing season. It seems not unlikely, however, that the rainfall distribution during a number of months preceding the beginning of a season's growth may have a measurable influence upon the width of the annual ring. There is a practical initial limit to the period, however, beyond which the influence of rainfall upon the current season's growth must become negligible. In the first investigations the initial limit was set at August 1 of the year preceding growth of the annual ring. The ending limit, which is taken as of August 31 of the year of growth, is rather definitely fixed by the time of year that the tree has completely formed the annual ring. The period taken is therefore 13 months.

For purposes of the following discussion the term "regression function" is defined as the function which expresses the influence of 1 additional centimeter of rainfall during a time interval upon that part of the width of the annual ring that is independent of the differences between trees and changes in age.[5] It is denoted by $\psi(t)$ where t is a

[5] If to the data of the last column of table 1 were added the mean width of the annual rings of the 12 trees during the 63 years (177.9 in hundredths of millimeters), the results would be widths of annual ring adjusted for differences between trees and changes in age. These are the values referred to by the term "width of annual ring" or "growth."

date within the 13-month period. Now there may be determined from the rainfall record (daily, weekly, monthly) during the 13-month period, another function, $\phi(t)$, to express the distribution of rainfall during the period, from which the rainfall in any time interval $t_k - t_i$ within the period would be given by

$$\int_{t_i}^{t_k} \phi(t) \cdot dt.$$

Because the influence of 1 cm of rainfall on the width of annual ring may be expressed by the regression function $\psi(t)$ during the same time interval, the influence of the rainfall distribution on growth during this special time interval would simply be

$$\int_{i}^{t_k} \psi(t) \cdot \phi(t) \cdot dt.$$

If this integral is calculated over the entire 13-month period (the initial and final limit of which may be designated as $-\frac{T}{2}$ and $\frac{T}{2}$) the whole effect of rainfall during the particular period upon radial growth may be written

$$\text{Growth} = V = M + \int_{-\frac{T}{2}}^{+\frac{T}{2}} \psi(t) \cdot \phi(t) \cdot dt,$$

M being a constant. This equation may be written for as many years as there are annual ring widths of the trees and 13-month rainfall distributions with which ring width is paired, the function $\psi(t)$ representing the average effect of 1 cm of rainfall to a unit of time (a day, a week, or a month) within the period, the unit of time being the same in all years.

For the years 1, 2, 3, ---------- n, we then have

$$\begin{aligned} V_1 &= M + \int_{-\frac{T}{2}}^{+\frac{T}{2}} \phi_1(t) \cdot \psi(t) \cdot dt \\ V_2 &= M + \int_{-\frac{T}{2}}^{+\frac{T}{2}} \phi_2(t) \cdot \psi(t) \cdot dt \qquad (2) \\ V_n &= M + \int_{-\frac{T}{2}}^{+\frac{T}{2}} \phi_n(t) \cdot \psi(t) \cdot dt \end{aligned}$$

Putting for M the arithmetic mean of the ring widths V_1, V_2, ---------- V_n, and letting $v_1 = (V_1 - M)$, $v_2 = (V_2 - M)$, -------- $v_n = (V_n - M)$, equations (2) become

$$\begin{aligned} v_1 &= \int_{-\frac{T}{2}}^{+\frac{T}{2}} \phi_1(t) \cdot \psi(t) \cdot dt \\ v_2 &= \int_{-\frac{T}{2}}^{+\frac{T}{2}} \phi_2(t) \cdot \psi(t) \cdot dt \qquad (3) \\ v_n &= \int_{-\frac{T}{2}}^{+\frac{T}{2}} \phi_n(t) \cdot \psi(t) \cdot dt \end{aligned}$$

It is necessary, therefore, to determine first the coefficients of the rainfall distribution, $\phi(t)$, for each of the 63 periods for which the precipitation record is at hand, and then the coefficients of the regression function, $\psi(t)$, such that the standard error of estimate of the n equations (3) is a minimum.

The first step toward the solution of this problem is the choice of mathematical form of the functions $\phi(t)$ and $\psi(t)$. As to $\phi(t)$ it is known that the distribution of rainfall during each of the periods of 13 months is a waved curve. Knowing nothing else regarding it, the simplest function expressing such curves is the polynomial

$$\phi(t) = c_0 + c_1 t + c_2 t^2 + c_3 t^3 + \cdots\cdots\cdots + c_k t^k.$$

Concerning the regression function, $\psi(t)$, it is known only that it must be a slowly changing function; a dry period of several days, for example, should influence diameter growth but little since the stems and the soil would contain enough water to balance the deficit for such a short period, It may, therefore, be expressed in the same form as $\phi(t)$, that is,

$$\psi(t) = \gamma_0 + \gamma_1 t + \gamma_2 t^2 + \cdots\cdots\cdots + \gamma_k t^k.$$

Following the inquiries of Fisher (*6*), these rational polynomials may be written in the form of orthogonal polynomials. The development below will show not only that such transformations enable us to determine the necessary coefficients, but that other important advantages follow. Computations are very much reduced since orthogonal functions have been tabulated by different authors. Perhaps the most convenient tables are those of Lorenz (*11*), who presents an excellent discussion of the properties of orthogonal polynomials and the calculation of their coefficients. Only their application to the present problem will be explained here.

A polynomial of rational function

$$y = c_0 + c_1 x^1 + c_2 x^2 + \cdots\cdots\cdots + c_k x^k$$

may be written in the form of an orthogonal polynomial

$$y = a_0 + a_1 X_1 + a_2 X_2 + \cdots\cdots\cdots + a_k X_k,$$

in which a_0, a_1, a_2, $\cdots\cdots a_k$ are constants; and X_i, which is a function of x that may be written $X_i(x)$, is defined by the following determinant:

$$X_i = b_i \begin{vmatrix} x^0 & x^1 & x^2 & \cdots\cdots & x^i \\ \Sigma x^0 & \Sigma x^1 & \Sigma x^2 & \cdots\cdots & \Sigma x^i \\ \Sigma x^1 & \Sigma x^2 & \Sigma x^3 & \cdots\cdots & \Sigma x^{i+1} \\ \Sigma x^2 & \Sigma x^3 & \Sigma x^4 & \cdots\cdots & \Sigma x^{i+2} \\ \Sigma x^{i-1} & \Sigma x^i & \Sigma x^{i+1} & \cdots\cdots & \Sigma x^{2i-1} \end{vmatrix}$$

Properties which make these functions orthogonal are the following, in which $i \neq k$:

$$\Sigma X_i = 0 \quad \text{or} \quad \int_{x=-\frac{T}{2}}^{\frac{T}{2}} X_i \cdot dx = 0$$

$$\Sigma X_i \cdot X_k = 0 \quad \text{or} \quad \int_{x=-\frac{T}{2}}^{\frac{T}{2}} X_i \cdot X_k \cdot dx = 0 \tag{4}$$

$$\frac{1}{N}\Sigma X^2{}_i = 1 \quad \text{or} \quad \int_{x=-\frac{T}{2}}^{\frac{T}{2}} X^2{}_i \cdot dx = 1$$

The constant b_i in the determinant is so chosen that $\Sigma X^2{}_i = N$, where N is the number of equidistantly spaced observations of x.

From the coefficients a_0, a_1, a_2, ---- a_k, of an orthogonal polynomial, the corresponding coefficients c_0, c_1, c_2, ---- c_k, of the polynomial of rational functions may be calculated, if needed. The latter, however, are not necessary to the present analyses.

The application of orthogonal polynomials in the present investigation consists of the following steps:

(1) The expression of the rainfall distribution within each time interval of each of the 13-month periods by the orthogonal polynomial

$$\phi(t) = a_0 + a_1 X_1 + a_2 X_2 + \text{----------} + a_k X_k \tag{5}$$

for which the determination of a_0, a_1, a_2, ---- a_k will be illustrated in the next section.

(2) The calculation of the regression function, which is to be expressed by another orthogonal polynomial

$$\psi(t) = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \text{----------} + \alpha_k X_k \tag{6}$$

for which the method of determining α_0, α_1, α_2, ---- α_k, will next be discussed.

According to equations (5) and (6) we have

$$v_i = \int_{-\frac{T}{2}}^{+\frac{T}{2}} \psi(t) \cdot \phi(t) \cdot dt = \int_{-\frac{T}{2}}^{+\frac{T}{2}} (a_0 + a_1 X_1 + \text{----------} a_k X_k)(\alpha_0 + \alpha_1 X_1 + \text{----------} \alpha_k X_k) \cdot dt$$

whence, on account of properties expressed by equations (4), in which, when $i \neq k$,

$$\int_{-\frac{T}{2}}^{+\frac{T}{2}} X_i \cdot X_k \cdot dt = 0 \text{ and } \int_{-\frac{T}{2}}^{+\frac{T}{2}} X^2{}_i \cdot dt = T,$$

it follows that

$$v_i = T(\alpha_0 a_0 + \alpha_1 a_1 + \alpha_2 a_2 + \text{----------} + \alpha_k a_k) + C \tag{7}$$

where C is the constant of integration.[6]

[6] Footnote on opposite page.

After we have obtained the rainfall coefficients $a_0, a_1, \ldots\ldots\ldots a_k$, for each of the sixty-three 13-month periods, we may set up 63 observation equations of (7), the unknowns, $\alpha_0, \alpha_1, \ldots\ldots\ldots \alpha_k$, of which are the coefficients of the regression function $\psi(t)$. And these may be calculated by the method of least squares. The multiple correlation coefficient obtained at the same time would then afford a measure of relationship between growth and rainfall distribution. Thereafter, upon multiplying the α_t coefficients by the corresponding functions X_t of equation (6) we obtain the regression function $\psi(t)$ which gives the effect of 1 additional centimeter of rainfall per month upon the width of the annual ring.

RAINFALL COEFFICIENTS AND THEIR PROGRESSIVE CHANGES

The rainfall distribution coefficients for each of the periods might be calculated from the rainfall record according to whatever time interval—daily, weekly, monthly—the record is preserved. Little, though, if anything, would be gained by using an interval which from phenological observations is less than that between calendar dates which mark the earliest activity of the buds in spring. Roughly, this interval is 2 weeks. The record of rainfall at hand, however, is according to successive months only. The span of a month seems long, and the rainfall distribution coefficients may, in consequence, not be as accurate as might be wished.

The first polynomials were fitted to the monthly precipitation values for a 13-month period beginning with August 1 of the year preceding growth and ending with August 31 of the year of growth in each of the 63 years. These were of the sixth degree. The calculation of $a_0, a_1, \ldots\ldots\ldots a_6$, for the annual-ring year 1924, is illustrated in table 5. The symmetrical arrangement in equal time intervals, t, measured from the median interval, is essential if the tables of orthogonal functions prepared by Lorenz (*11*) are used. The form of table 5 (known as the scheme of Chetverikoff) is that suggested by Lorenz when N is odd. The coefficients $a_0, a_1, a_2, \ldots\ldots\ldots a_6$ are computed through the use of cumulative sums, combined with certain parameters which have been computed by Lorenz (*11*), by whom the technique of these computations is fully described and the necessary tables given.

The coefficients $a_0, a_1, a_2, \ldots\ldots\ldots a_6$ are those of the orthogonal polynomial equation (5), according to the method of least squares. An immediate advantage of the orthogonal polynomial form is that coefficients up to a given degree are not affected if coefficients of higher degree are added. By simply adding the term a_3X_3 to the polynomial of second degree we obtain the polynomial of third degree, or by dropping the a_2X_2 term, the remainder is the polynomial of first degree.

---

[6] Certain remarks concerning C and T may prove helpful:
The constant of integration must be $C=-T\,(\alpha_0\delta_0+\alpha_1\delta_1+\ldots\ldots\ldots+\alpha_k\delta_k)$ where $\delta_0, \delta_1, \ldots\ldots\ldots \delta_k$ are the means of the corresponding rainfall coefficients of the n years considered. These mean coefficients characterize the average rainfall distribution of the n years, and consequently the deviation of growth $\bar{v}_t$ for average rainfall conditions should be zero, because the sum of the n equations (3)=0. It follows necessarily that

$$\bar{v}_t=T\,(\alpha_0\delta_0+\alpha_1\delta_1+\ldots\ldots\ldots+\alpha_k\delta_k)+C=0,$$

if C is the quantity given above.
Since T is the entire time period under consideration (in this case, 13 months), and since the rainfall coefficients, $a_0, a_1, a_2, \ldots\ldots\ldots a_k$, are calculated on the basis of rainfall data obtained for a certain number, N, of time intervals ΔT (using the functions X_t corresponding to this number of observations N), T must be $N\cdot\Delta T$. In this discussion $\Delta T=1$ month, hence $T=N\cdot\Delta T=13$.

TABLE 5.—*Scheme of Chetverikoff for calculation of rainfall coefficients, annual ring year 1924* [1]

Month	t	w								
1923										
August	−6	51	51	51	51	51	51	51	51	51
September	−5	80	131	182	233	284	335	386	437	
October	−4	227	358	540	773	1,057	1,392	1,778	2,215=s_{-5}	
November	−3	141	499	1,039	1,812	2,869	4,261=s_{-4}	6,039=s_{-3}		
December	−2	172	671	1,710	3,522=s_{-2}	6,391=s_{-3}				
1924										
January	−1	38	709=s_{-0}	2,419=s_{-1}						
February	0	26	26=w_0							
March	1	55	717=s_0	2,596=s_1	6,797=s_2					
April	2	138	662	1,879	4,201	8,130=s_3	14,258=s_4			
May	3	153	524	1,217	2,322	3,929	6,128	9,009=s_5	12,662=s_6	
June	4	139	371	693	1,105	1,607	2,199	2,881	3,653	
July	5	142	232	322	412	502	592	682	772	
August	6	90	90	90	90	90	90	90	90	
			$s_{-0}+w_0+s_0=S_0$	$s_1-s_{-1}=S_1$	$s_2+s_{-2}=S_2$	$s_3-s_{-3}=S_3$	$s_4+s_{-4}=S_4$	$s_5-s_{-5}=S_5$	$s_6+s_{-6}=S_6$	
S_i			1,452	177	10,319	1,739	18,519	2,970	14,877	
a_i			a_0=111.692	a_1=3.639	a_2=0.825	a_3=11.956	a_4=−42.691	a_5=−0.058	a_6=24.155	

[1] w=rainfall in millimeters. N=13.

Upon combining the coefficients of table 5 with the corresponding orthogonal functions, as tabled in Lorenz's paper (*11*), the values of

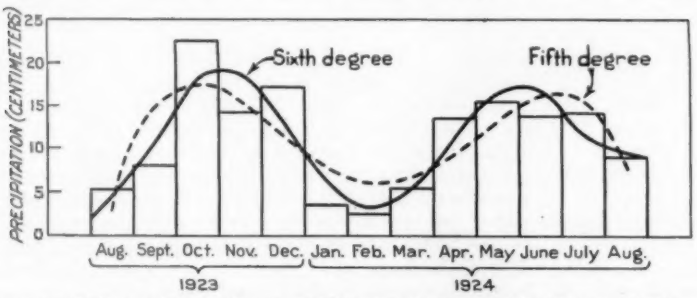

FIGURE 4.—Monthly precipitation from August 1923 to August 1924 compared with the polynomial curves of fifth and sixth degree.

the precipitation curve are at the points corresponding to the months for the annual-ring year 1924. In figure 4 the curves of both the fifth and sixth degrees are presented. As may be checked in table 5, the first coefficient, a_0, is simply the arithmetic mean of the monthly rainfall records. The second coefficient, a_1, leads to the average change of rainfall per month during the period, the third coefficient, a_2, measures the parabolic term, and so on as more complex features of the precipitation distribution are taken into account.

The coefficients for the 13-month periods are listed in table 6, and they are presented graphically, by 7-year moving averages, in figure 5. Aside from whatever bearing these coefficients may have on tree growth, they are of interest in themselves in that the moving averages of figure 5 suggest systematic changes in their values during the 63 years. In order to test whether these are significant, a polynomial of sixth degree, of the same form as equation (5), was fitted to the 63 observed values of each coefficient of table 6. These, in turn, are listed in table 7, the first line of which—the a'_0 coefficients—are the averages of a_t from the corresponding column of table 6.

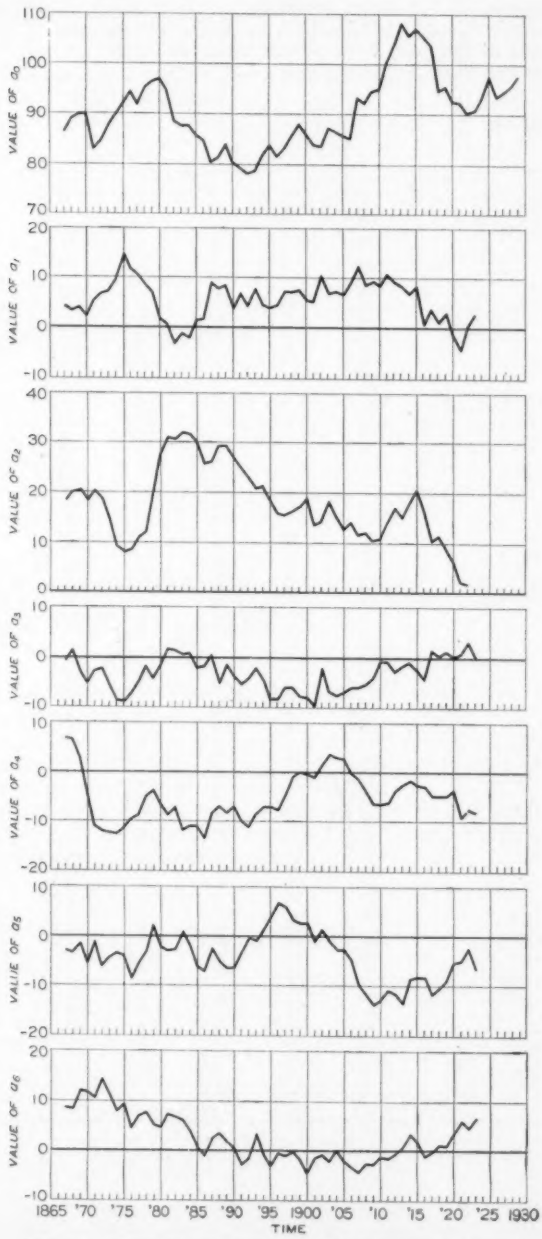
VALUE OF a_0
VALUE OF a_1
VALUE OF a_2
VALUE OF a_3
VALUE OF a_4
VALUE OF a_5
VALUE OF a_6
1865 '70 '75 '80 '85 '90 '95 1900 '05 '10 '15 '20 '25 1930
TIME

FIGURE 5.—Polynomial coefficients according to 7-year moving averages.

TABLE 6.—*Coefficients of orthogonal polynomials which express precipitation trends (in millimeters) by monthly intervals from August of a preceding year to August inclusive of a current year, for the current years 1864–1926*

Current year	Coefficients for—						
	a_0	a_1	a_2	a_3	a_4	a_5	a_6
1864	80	−3	10	−16	−17	−1	14
1865	73	22	14	12	14	0	−4
1866	100	24	27	−1	39	12	21
1867	118	−13	9	−28	24	−28	16
1868	71	−3	16	4	−5	4	−13
1869	88	3	13	12	−8	−1	19
1870	74	−2	38	12	3	−5	8
1871	94	−11	22	0	−19	−5	12
1872	84	28	17	−18	−12	12	21
1873	100	11	12	−17	−14	−15	20
1874	70	9	22	−11	−23	2	6
1875	81	8	5	8	−12	−30	14
1876	111	6	−15	−15	−12	10	−2
1877	89	14	1	−8	2	3	−15
1878	110	27	14	−2	−11	−8	15
1879	101	7	21	−7	2	−22	−5
1880	80	2	27	0	−8	7	36
1881	94	−5	31	12	4	20	12
1882	91	−2	61	−10	−2	7	−4
1883	114	−30	37	4	−34	−20	−6
1884	73	6	24	14	−12	−2	3
1885	65	−1	12	−22	0	−7	11
1886	95	20	31	7	−30	1	−9
1887	81	−1	24	1	−3	−14	18
1888	80	18	19	−8	5	−9	−9
1889	86	1	31	−7	−20	1	−18
1890	81	20	41	19	2	12	20
1891	81	−2	46	−24	−3	−23	12
1892	84	4	13	2	−10	−15	−2
1893	69	−13	14	−8	−20	2	−19
1894	74	20	9	−11	−25	10	−3
1895	73	0	7	0	−4	11	0
1896	90	25	16	7	0	−3	17
1897	102	−2	44	2	13	29	−10
1898	94	−5	26	−51	−4	−4	−5
1899	69	6	6	1	−13	2	16
1900	80	6	12	9	1	−2	−22
1901	92	19	14	−10	1	−8	2
1902	88	3	15	−12	3	5	−10
1903	74	11	28	5	−2	−3	−3
1904	90	−5	7	−11	6	4	10
1905	92	31	31	3	14	13	4
1906	94	−18	22	−31	3	−16	3
1907	75	11	9	3	−2	−14	−6
1908	88	13	4	−6	−4	−9	−13
1909	84	20	24	−5	−17	−8	−18
1910	130	26	10	4	−10	−39	−11
1911	84	−24	10	−6	−11	−9	22
1912	108	36	19	12	−5	−2	4
1913	97	−22	25	−8	3	−9	13
1914	122	27	14	2	0	1	−8
1915	103	2	17	−18	13	−14	−7
1916	114	13	11	3	−8	−22	−8
1917	113	16	30	9	−4	−4	8
1918	93	−15	30	−18	−17	−8	10
1919	97	−14	−12	0	−7	−3	−15
1920	84	−2	−15	33	−11	−32	19
1921	60	7	20	−6	−1	9	2
1922	108	14	−1	−12	14	−2	−8
1923	93	−23	−6	−4	−1	3	11
1924	112	4	1	12	−43	0	24
1925	79	17	26	0	−6	9	−4
1926	100	2	0	−21	−10	−32	2
Mean	90.1	5.5	16.7	−3.5	−4.9	−4.1	3.0

TABLE 7.—*Coefficients of orthogonal polynomial equations which express trends of the a_i coefficients of table 6, during the 63 years 1864–1926*

Coefficient	Coefficients of polynomial equation for the following coefficients from table 6						
	a_0	a_1	a_2	a_3	a_4	a_5	a_6
a'_0	90.100	5.500	16.700	−3.500	−4.900	−4.100	3.000
a'_1	3.142	−.605	−3.586	.322	−.789	−1.821	−2.663
a'_2	2.518	−.743	−3.983	.743	.955	−1.248	3.125
a'_3	−.742	−1.259	1.101	.334	−4.708	.298	1.257
a'_4	−4.503	−.040	.572	−1.455	.993	2.125	−.885
a'_5	−.043	1.626	−2.070	−1.936	.242	−.328	.078
a'_6	2.765	1.507	.063	−1.129	−.998	−2.429	−.509

The test is carried out through the analysis of variance of each a_i coefficient of table 6. In general,

$$\Sigma(y-\bar{y})^2=\Sigma(y-Y)^2+\Sigma(Y-\bar{y})^2$$

in which y is a dependent variate, $\bar{y}$ is the arithmetic average of these variates, and Y is the trend value of y as estimated by the method of least squares. In the analysis of variance of a_0 as an illustration, the left-hand member of the above equation is the sum of the squares of the 63 deviations of a_0 from their mean, as computed from the values of the a_0 column of table 6. It may be written

$$\Sigma(a_0-a'_0)^2=\Sigma a^2{}_0-63a'^2{}_0,$$

in which a'_0 is the arithmetic average of the 63 observations of a_0. The term corresponding to $\Sigma(Y-\bar{y})^2$ may, on account of the orthogonality of equation (5), be simplified to

$$63(a'^2{}_1+a'^2{}_2+a'^2{}_3+a'^2{}_4+a'^2{}_5+a'^2{}_6),$$

obviating the need of calculating the trend values of a_0 (*11, pp. 44–45*). The sum of squares independent of the trend $[\Sigma(y-Y)^2]$ is then the difference between the total sum of squares $[\Sigma(y-\bar{y})^2]$ and the sum of squares due to the trend $[\Sigma(Y-\bar{y})^2]$. Calculated thus, the sums of squares for the analyses of variance of a_0, a_1, a_2,--------a_6, and their degrees of freedom, are given in the second, third, and fourth columns of table 8; mean squares and the observed values of Snedecor's (*15*) ratio F are in the last three columns.

TABLE 8.—*Analyses of variance of rainfall coefficients*

Coefficient	Sum of squares and degrees of freedom in—			Mean square in—		F
	Trend, 6 degrees	Deviations from trend, 56 degrees	Total, 62 degrees	Trend	Deviations from trend	
a_0	2,816.1	11,428.2	14,244.3	469.35	204.08	[1] 2.30
a_1	466.2	12,379.5	12,845.7	77.70	221.06	[2] 2.85
a_2	2,198.7	11,648.7	13,847.4	366.45	208.01	[1] 1.76
a_3	497.7	10,558.8	11,056.5	82.95	188.55	[2] 2.27
a_4	1,625.4	8,964.9	10,590.3	270.90	160.09	[1] 1.69
a_5	976.5	9,651.6	10,628.1	162.75	172.35	[2] 1.06
a_6	1,165.5	9,261.0	10,426.5	194.25	165.38	[1] 1.17

[1] F=2.27 at 5-percent level of significance.
[2] F=3.15 at 5-percent level of significance.

From the distribution of F in random samples when based on 6 and 56 degrees of freedom as in this investigation, the value of F at the 5-percent level of significance is 2.27, provided the greater mean

square is based on 6 degrees of freedom, and 3.15 if based on 56 degrees of freedom (*15, table 25*). The observed F for a_0 is the only one that exceeds expectation at this level of significance. With this single exception, then, it appears that there is no real evidence of systematic changes of the rainfall coefficients during the 63 years with which this investigation is concerned. In successive years a given coefficient—other than a_0—seemingly takes random values from a distribution whose mean and standard deviation are given in table 9. The mean and standard deviation of a_0 are also listed in the table. The mean of each coefficient is taken from the first line of table 7 and the standard deviation is the square root of $\frac{1}{62}$ times the sum of squares of the totals in table 8.

TABLE 9.—*Means and standard deviations of the rainfall coefficients, 13-month periods*

	a_0	a_1	a_2	a_3	a_4	a_5	a_6
Mean	90.100	5.500	16.700	−3.500	−4.900	−4.100	3.000
Standard deviation	15.157	14.394	14.945	13.354	13.069	13.093	12.962

The mean values of these coefficients differ significantly from zero owing to the variations of monthly rainfall within the period. They represent the average sequence of rainfall, presented graphically in figure 6 in comparison with the actual monthly averages.

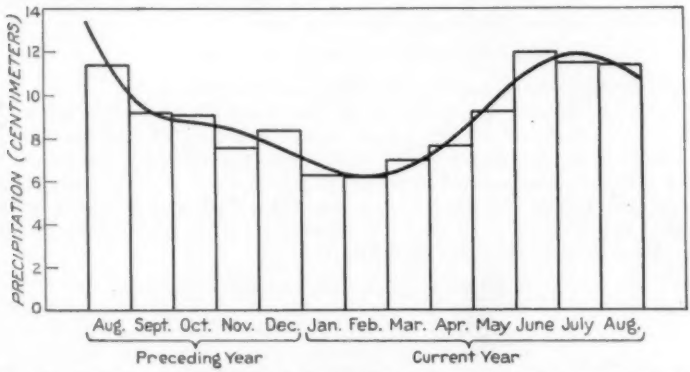

FIGURE 6.—Average monthly precipitation from August to August, inclusive, and its representation by the average of the polynomial coefficients to degree 6.

INVESTIGATIONS PRELIMINARY TO THE REGRESSION OF GROWTH ON RAINFALL

It has often been observed that two variables, known to be intrinsically independent of each other, are apparently correlated as a result of parallel progression of their observed values in time. Such relationships are known as spurious, or nonsense, correlations (*2, pp. 287–289*). An estimate of the true correlation between variables which are undergoing progressive changes in time may be calculated from the residuals from secular trends, provided the trend curves successfully eliminate secular changes. It must be emphasized that

the true form of secular trends is not usually known. While a trend curve of polynomial form may be fitted to time series with any desired degree of approximation by simply increasing the degree of polynomial, yet the decision as to the sufficiency of a given degree is not universally and entirely based on objective analyses, even though complete use be made of statistical checks. The decision is, rather, a common sense one, in that intimate acquaintance with the data under investigation is a most important consideration in the judgment as to whether a given trend is reasonable.

Attention is called to these difficulties because the following discussion goes beyond the results of mechanical application of certain well-known statistical formulae into a realm that may not be entirely free from the idiosyncrasies of individual judgments.

It was pointed out in connection with table 8 that the trend of the rainfall coefficients a_0—the average monthly rainfall from August of a preceding year to August inclusive of a current year—could hardly have been the outcome of chance fluctuations of annual rainfall during the 63 years. Consequently the question arises as to whether the best estimate of the effect of 1 additional centimeter of rainfall per month on tree growth is to be obtained from the observed rainfall coefficients a_0, or from the residuals of a_0 from its secular trend. The tree data also, while they are already averages of residuals from separate trends designed to eliminate progressive changes peculiar to each of the 12 trees, may still be subject to collective progression, common to all the trees in the stand; such, for instance, as may be due to changes in soil conditions over the 63-year period.

In order to eliminate progressive changes both in the tree-ring widths and in the rainfall coefficients a_0, trend curves as expressed by orthogonal polynomials of degree 1 to 8 were fitted to the annual observations of both variables, rendering unnecessary the tedious computations of the 8×8 sets of residuals of each variable upon which the direct calculation of the correlation coefficients would be based. These correlation coefficients may be expressed

$$r_{kk'}=\frac{\Sigma y_k y'_{k'}}{\sqrt{\Sigma y^2{}_k \times y'^2{}_{k'}}} \tag{8}$$

in which y is a residual of an average annual ring width from trend of degree k [$k=0, 1, 2, - - - - - 8$] as indicated by its subscript, and y' is a residual of average monthly rainfall during the 13-month period from trend of degree k' [$k'=0, 1, 2, - - - - - 8$] also indicated by its subscript. But following Lorenz (*11, pp. 49–50*) the calculation of the correlation coefficients may be greatly shortened, for equation (8) may be written

$$r_{kk'}=\frac{\Sigma y_0 y'_0-n(a_0a'_0+a_1a'_1+ - - - - - +a_ka'_k)}{\sqrt{[\Sigma y_0{}^2-n(a_0{}^2+a_1{}^2+ - - - - - +a_k{}^2)][\Sigma y'_0{}^2-n(a'_0{}^2+a'_1{}^2+ - - - - - +a'_{k'}{}^2)]}} \tag{9}$$

in which $a_0, a_1, - - - - - a_k$ are the coefficients of the orthogonal polynomial of degree k, fitted to 63 average annual ring widths y, and $a_0', a_1', - - - - - a'_{k'}$ are the coefficients of the orthogonal polynomial of degree k', fitted to the 63 periods of 13-monthly rainfall averages.

These coefficients have already been calculated as steps in their respective polynomials. The only new computation is the single sum of the products ($y_o\ y'_o$) common to all the correlation coefficients discussed below. The numerator of equation (9) for a correlation coefficient between residuals from trends of unequal degree is carried out to the higher of the two degrees, while the terms in the denominator are taken to the degree of the trend with which the residuals are identified. Writing r_{02} as an illustration,

$$r_{02}=\frac{\Sigma y_0 y_0' - n(a_0 a_0' + a_1 a_1' + a_2 a_2')}{\sqrt{[\Sigma y_0^2 - n a_0^2][\Sigma y_0'^2 - n(a_0'^2 + a_1'^2 + a_2'^2)]}}$$

Using equation (9) the correlation coefficients are as listed in table 10. The first column and first line of correlation coefficients are of particular interest, the first column containing those between mean rainfall (i. e., residuals from trend of degree 0) and the average residuals of annual ring width of the 12 trees from trends of degree 0 to 8. The highest correlation coefficient in this column is r_{30}, between ring width residuals from trend of degree 3 to rainfall, the value of which is

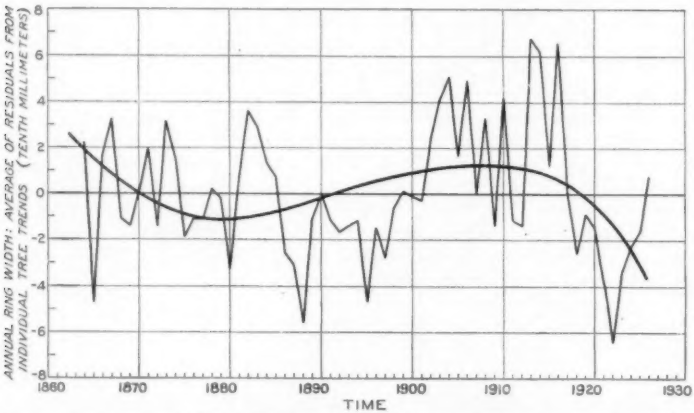

FIGURE 7.—Annual ring width data and their secular trend as represented by the polynomial of degree 3.

0.367; between residuals of ring width from trends of higher degree than 3 and rainfall, the correlation coefficients fall off to 0.243 for r_{80}. Evidently trend curves of degree higher than 3 cannot be regarded as eliminating only the secular changes in tree growth; they also eliminate part of the annual growth fluctuation that is dependent on rainfall, as indicated by the generally declining values of the coefficients from r_{40} to r_{80}. On the other hand, if one may judge by the increasing correlation coefficients between rainfall and annual ring width residuals from trends up to degree 3, these lower trends eliminate systematic changes in ring width that have nothing to do with rainfall; hence the annual ring width residuals from trend of degree 3 are probably the best values for use in the regression function. The annual ring width data and the third-degree trend fitted thereto are presented in figure 7.

There are analogous conditions in the first line of table 10. The correlation coefficient r_{02}—between annual ring width and rainfall residuals from trend of degree 2—is the highest with a value of 0.374, dropping gradually to 0.218 in r_{08}. Through the trends of degree higher than 2, rather short fluctuations in annual rainfall are eliminated, and these evidently affect annual growth. In the light of all the correlation coefficients in the table, it seems at first that the best estimate of the effect of an added centimeter of rainfall on tree growth should be obtained from the correlation between the residuals of annual ring width from trend of degree 3 and the residuals of the a_0 coefficients from trend of degree 2. However, since the increase in the correlation coefficient r_{30} [=0.367] over r_{00} [=0.348] is considerably greater than of r_{32} [=0.380] over r_{02} [=0.374], but little would be gained through the use of residuals of the rainfall values a_0 from trend, rather than the a_0 values themselves. For it seems likely that the trend of degree 2 through the rainfall coefficients a_0 eliminates part of the rainfall which has influenced tree growth. Furthermore, spurious correlation is avoided if the secular trend of but one of the two variables is eliminated; hence the original rainfall values are used for the independent variable a_0.

TABLE 10.—*Correlation coefficients between residuals of annual ring width and of annual rainfall, taken from trends of degree 0 to 8*

Annual ring width variable: residuals from trend of degree indicated	Rainfall variable: residuals from trend of degree indicated								
	0	1	2	3	4	5	6	7	8
0	0.348	0.355	0.374	0.357	0.299	0.275	0.234	0.219	0.218
1	.347	.355	.374	.357	.299	.275	.234	.219	.218
2	.362	.370	.375	.358	.300	.301	.235	.219	.219
3	.367	.375	.380	.381	.319	.320	.250	.233	.233
4	.302	.309	.314	.314	.330	.331	.259	.241	.241
5	.311	.318	.323	.323	.340	.340	.266	.248	.248
6	.260	.266	.269	.270	.284	.284	.290	.270	.270
7	.243	.249	.252	.253	.266	.266	.271	.274	.274
8	.243	.248	.252	.252	.265	.265	.271	.274	.274

REGRESSION OF GROWTH ON RAINFALL AT DIFFERENT SEASONS

As an outcome of the analyses just discussed, two regression equations were calculated, each expressing the average influence of the rainfall coefficients a_0, a_1,----------a_5 for the 13-month periods on annual ring width. The a_6 coefficient was not used as an independent variable because it seemed likely that the others should suffice to describe the influence of precipitation adequately. The observations upon which these regression equations are based differ only in the values of annual ring width. In the first, the annual ring width residuals from trend of degree zero—the values of table 1, denoted by the symbol $v^{(0)}$—were taken; in the second, the residuals were taken from trend of degree 3—that is, the deviation of the values of table 1, last column, from the trend curve of figure 7, denoted by $v^{(3)}$.

Observation equations of the form of equation (7) in which

$$v^{(0)}=T(\alpha_0 a_0+\alpha_1 a_1+\text{----------}+\alpha_5 a_5)$$

were written down for each of the 63 years, the $v^{(0)}$ values for which were taken from table 1 and the values of the a_t rainfall coefficients

from table 6 for corresponding years. These equations lead to the normal equations, which when solved simultaneously, render 13—since $T=13$—times the coefficients α_i of the regression function. Then upon putting the α_i coefficients in equation (6) in which

$$\psi(t)=\alpha_0+\alpha_1X_1+\alpha_2X_2+\text{----------}+\alpha_5X_5,$$

where the functions X_i are taken from Lorenz's tables (*11*), we have, finally, the expression of the regression function $\psi(t)$. This is presented graphically in figure 8.

In like manner, the residuals of annual ring width from trend of degree 3 lead to the regression function $\psi(t)$ of $v^{(3)}$; and this is also presented in figure 8.

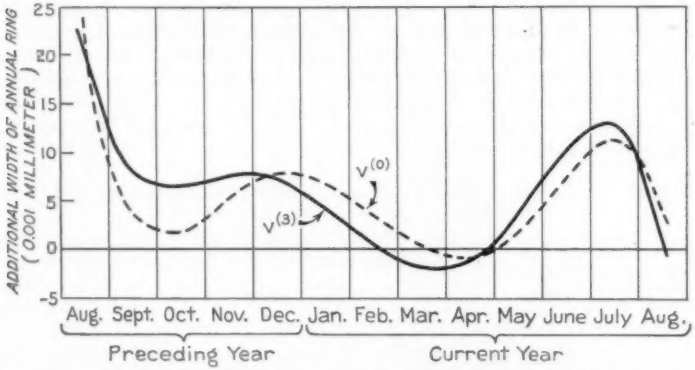

FIGURE 8.—Average effect of 1 additional centimeter of precipitation per month on width of annual ring (13-month period). The curve marked $r^{(0)}$ is based on the annual ring width data of table 1 without further adjustment, while that marked $r^{(3)}$ is based on deviations of the same data from the trend curve of figure 7.

The two regression curves are quite similar. With the exception of March and April, an extra centimeter of precipitation per month is beneficial to the growing tree, although with varying efficiency in the different seasons of the period. The most striking feature, common to both curves of figure 8, is that the efficiency of an added centimeter of precipitation during the months of the year preceding growth is apparently as great as that during the season of growth itself, a higher-than-average precipitation during the late summer of the preceding year contributing most to the current season's growth. During winter the efficiency of extra precipitation falls off, perhaps because it is largely in the form of snow, and scarcely available, if at all, before the spring thaw, for in early spring the curves reach a minimum. They then pick up steadily through May and June, reaching a maximum in July. Thereafter they decline with the completion of the season's growth.

The negligible influence of extra precipitation during August on the current season's growth, as contrasted with its relatively great influence on next year's growth, suggests that August precipitation, and perhaps that of the earlier summer as well, is especially stimulating to the production of food reserves needed for next year's growth. But as the regression curves of figure 8 hang loose-ended relatively

high above zero in August of the preceding year, the 13-month period apparently does not describe the influence of precipitation on width of annual ring with sufficient completeness.

It was for this reason that the rainfall coefficients and the regression function were finally calculated for the 15-month period, thus including the June and July precipitation of the preceding year. Furthermore the a_6 rainfall coefficients for the 15-month periods were used as a seventh independent variable. Two regression curves—one for $v^{(0)}$ and one for $v^{(3)}$—were accordingly calculated, and these are presented in figure 9.

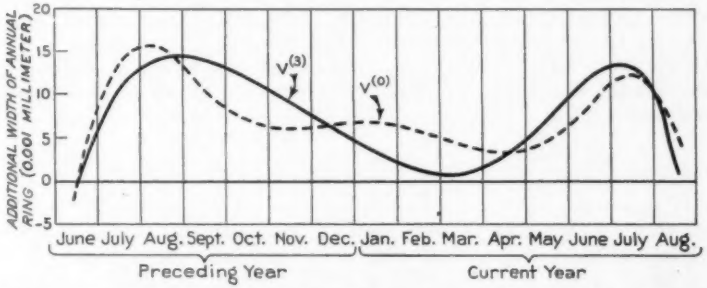

FIGURE 9.—Average effect of 1 additional centimeter of precipitation per month on width of annual ring (15-month period). The curve marked $v^{(0)}$ is based on the annual ring width data of table 1 without further adjustment, while that marked $v^{(3)}$ is based on deviations of the same data from the trend curve of figure 7.

This time, as anticipated, the regression curves decline toward zero on the left-hand end. Otherwise the general trends are much like those of the 13-month period. The area under the curves—particularly under $v^{(3)}$, which is based on annual-ring observations from which progressive changes not affected by precipitation have been eliminated—represents the relative efficiency of 1 additional centimeter of precipitation per month. The greater efficiency of additional precipitation from June to December of the year preceding the formation of the annual ring over that of the 8-month period of the current year is again apparent.

But perhaps the most interesting feature is brought out in figure 10, in which the curve of $v^{(3)}$ for the 15-month period is broken into two parts and set along the time scale of a year from approximately the initiation of one season's ring to that of the next. Curve *a* represents the influence of an additional centimeter of precipitation per month upon the growth of the current season's ring, while curve *b* represents its influence on next year's ring. The new point of interest is that the influence of extra precipitation on next year's growth becomes really pronounced at about the same time (early July) that its influence on this year's growth passes its maximum, and reaches its own maximum, in turn, at the end of this year's growing season. During the month of July, primarily, additional precipitation contributes to the current season's active growth, and at the same time apparently stimulates the building up of reserves in preparation for the spring get-away. From August on, the preparation for next season continues, although at a lessened rate.

No curve, however, may be regarded as representing exactly the true influence of the precipitation distribution on growth. As in all correlation analyses, the curves of figures 8 and 9 hold within certain limits of error only. Graphically the limits of acceptable error might be portrayed by a painted strip covering the two curves of each figure the trend of which should probably be guided mostly by the curve of $v^{(3)}$.

The sum of squares of the dependent variable is reduced in the proportion $\frac{1}{(1-R^2)}$ where R is the multiple correlation coefficient. But observations during 63 years only give 62 degrees of freedom in $v^{(0)}$ to start with; while in $v^{(3)}$ there are but 59 because 3 degrees of freedom have been eliminated by the trend of third degree, the residuals from which are $v^{(3)}$ by definition. At most, therefore, the samples are not large, particularly if the correlation coefficients are moderate.

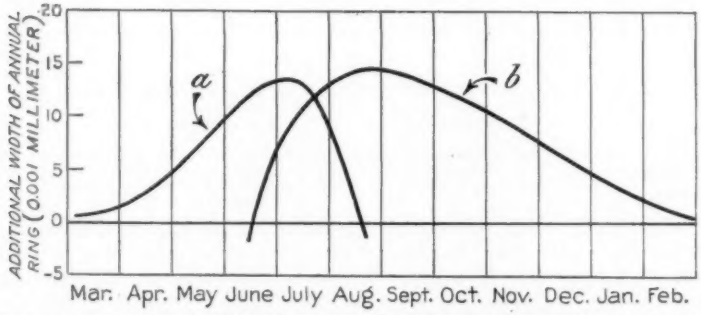

FIGURE 10.—Average effect of 1 additional centimeter of precipitation per month during 1 year on (*a*) the current annual ring and (*b*) next year's annual ring.

Table 11 contains the analyses of variance of the data based on the regression equations of the 13-month periods. The sum of squares of both $v^{(0)}$ and $v^{(3)}$ are divided into parts due to, and independent of, their respective regression equations, the former accounting for 6 degrees of freedom in each case. The correlation coefficient between actual and calculated values of $v^{(0)}$ is $\sqrt{\frac{10,168}{51,724}}=0.443$, indicating only moderate correlation between growth and the rainfall distribution. The ratio F of $v^{(0)}$ is $\frac{1,695}{742}=2.28$. From Snedecor's table (*15*) the value of F at the 5-percent level in the case of independent variates based on the same number of degrees of freedom as are available here, is 2.27. The regression is therefore adjudged significant.

The correlation coefficient between actual and calculated values of $v^{(3)}$ is $\sqrt{\frac{10,272}{45,342}}=0.476$ and the observed ratio F is $\frac{1,712}{662}=2.59$. For the same number of degrees of freedom the value of F at the 5-percent level in the case of independent variates is 2.28. This regression, consequently, is also judged significant.

TABLE 11.—*Analyses of variance based upon the regressions according to 13-month and 15-month periods*

13-MONTH PERIODS

Source of variation	Sum of squares	Degrees of freedom	Mean squares	Source of variation	Sum of squares	Degrees of freedom	Mean squares
Analysis of $v^{(0)}$:				Analysis of $v^{(3)}$:			
Regression	10,168	6	1,695	Regression	10,272	6	1,712
Deviation from regression	41,556	56	742	Deviation from regression	35,070	53	662
Total	51,724	62		Total	45,342	59	

15-MONTH PERIODS

Analysis of $v^{(0)}$:				Analysis of $v^{(3)}$:			
Regression	8,463	7	1,209	Regression	9,044	7	1,292
Deviation from regression	43,261	55	787	Deviation from regression	36,298	52	698
Total	51,724	62		Total	45,342	59	

Table 11 contains also the analyses of variance based on the 15-month periods. While this greater period length and the inclusion of the additional independent variable—the a_6 coefficient—are believed to supply a more complete description of the influence of rainfall on annual-ring width, a certain loss of significance results, if the latter is placed at the 5-percent level; for the value of F at this level, taken from Snedecor's table, is 2.19 for the 7 and 55 degrees of freedom for $v^{(0)}$, whereas the observed F is $\frac{1,209}{787}=1.54$. The corresponding values of F for the 7 and 52 degrees of freedom upon which the analysis of $v^{(3)}$ is based are 2.20 and 1.85. Several reasons, other than the fact that the size of sample is not large, may be suggested for the lack of positive proof of the calculated influence of the rainfall distribution during the 15-month periods on the timber represented by these data. Among them are the following: (1) The rainfall record according to intervals of 1 month may cover up part of the correlation that weekly or fortnightly records might bring out. (2) The trees from which the annual ring width measurements were taken grew at a distance of about 8 km from the weather station, so that the rainfall record used may not have been precisely that to which the trees were subjected. (3) In a region where the average monthly precipitation is 9 cm, rainfall distribution throughout the year is probably far from a limiting factor in timber growth.

SUMMARY

The annual ring widths from 1864 to 1926 of 12 dominant *Abies alba* sample trees taken from a 120-year-old stand of fir-spruce-beech, and rainfall records by monthly intervals for this period, were available for a study of the effect of climate on the magnitude of timber-growth fluctuation and an investigation of the influence of rainfall distribution on tree growth.

After plotting the annual ring width on the year of growth for each tree, trend curves of growth, of first or second degree only, were fitted

to the data of each tree in order to eliminate the effect of age on width of annual ring.

An average correlation coefficient of about 0.5 was found between residuals from trend of single trees, and of about 0.9 between average residuals of six trees from their trends, proving that an important source of variation of annual ring widths lies outside the forest. This source is taken to be the annual fluctuation of climate. The magnitude of variation of annual ring width due to fluctuation of climate as calculated through the analysis of variance is a standard deviation that is 15.4 percent of the average ring width of the 12 trees during the 63 years.

The effect of fluctuation in climate on basal area and volume growth are calculated, and a method of eliminating the effect of climate in comparing the effect of treatment, epidemic, or catastrophe on periodic growth is illustrated.

The rainfall by months during each of the sixty-three 13-month periods, between August 1 of the year preceding growth of annual ring and August 31 of the year of growth, was expressed by an orthogonal polynomial of sixth degree.

In order to determine whether progressive changes in rainfall distribution within the 13-month periods could be detected over the course of the 63 years under investigation, each of the coefficients was plotted on the year of its occurrence, and to each set of 63 values an orthogonal polynomial of degree 6 was, in turn, fitted. It was found that the a_o coefficient, which represents the average monthly rainfall during each 13-month period, was the only one which varied systematically over the 63 years.

As a means of eliminating possible progressive changes in annual ring width not associated with progressive changes in average monthly rainfall, orthogonal polynomials of several degrees were also fitted to the ring-width data over the 63 years, and the correlation between residuals from trend of these and residuals from trend of various degrees of the a_o rainfall coefficients were calculated. While the highest correlation coefficient existed between residuals from trend of degree 3 for annual ring width and from trend of degree 2 for the a_o coefficient, it was very little higher than the correlation coefficient between annual ring width residual from trend of degree 3 and the a_o coefficient residual from trend of degree zero. As a consequence it seemed that the most appropriate expression of the effect of rainfall distribution on annual ring width should be obtained from the regression of annual ring width residual from trend of degree 3 on the rainfall coefficients from which secular trends, if present, were not eliminated. This was accordingly done. For comparison, the regression equation of annual ring width, from which trend was not eliminated, on the rainfall coefficients was also calculated.

By means of tables of orthogonal functions these regression equations are made to express the average effect of an added centimeter of rainfall per month during the 13-month period on width of annual ring.

While the regressions based upon the 13-month period were significant, they did not seem to portray the influence of rainfall with sufficient completeness. Accordingly, the period length was extended to 15 months, thereby including the rainfall of June and July of the year preceding growth. A new set of rainfall coefficients of orthogonal

polynomials was calculated, and the width of annual ring expressed in terms of these. The results, showing satisfactory completeness, were not assuredly significant. Several reasons are suggested in explanation.

LITERATURE CITED

(1) BOMAN, A.
1927. TUTKIMUKSIA MÄNNYN PAKSUUSKASVUN MONIVUOTISISTA VAIHTELUISTA . . . (ÜBER VIELJAHRIGE SCHWANKUNGEN IM DICKENWACHSTUM DER KIEFER (PINUS SILVESTRIS), (REFERAT). Acta Forest. Fennica 32, 177 pp., illus. [Separately paged.] [In Finnish. German summary, 54 pp.]

(2) BRUCE, D., and SCHUMACHER, F. X.
1935. FOREST MENSURATION. 360 pp., illus. New York and London.

(3) CIESLAR, A.
1907. EINIGE BEZIEHUNGEN ZWISCHEN HOLZZUWACHS UND WITTERUNG. Centbl. Gesam. Forstw. 33: [233]–246, 289–311, illus.

(4) DILLER, O. D.
1935. THE RELATION OF TEMPERATURE AND PRECIPITATION TO THE GROWTH OF BEECH IN NORTHERN INDIANA. Ecology 16: 72–81, illus.

(5) DOUGLASS, A. E.
1928. CLIMATIC CYCLES AND TREE GROWTH; A STUDY OF THE ANNUAL RINGS OF TREES IN RELATION TO CLIMATE AND SOLAR ACTIVITY. v. 2, illus. (Carnegie Inst. Wash. Pub. 289.)

(6) FISHER, R. A.
1925. THE INFLUENCE OF RAINFALL ON THE YIELD OF WHEAT AT ROTHAMSTED. Roy. Soc. [London], Phil. Trans., Ser. B, 213: 89–142, illus.

(7) IRWIN, J. O.
1931. MATHEMATICAL THEOREMS INVOLVED IN THE ANALYSIS OF VARIANCE. Jour. Roy. Statis. Soc. 94: 284–300.

(8) KNUCHEL, H.
1933. ÜBER ZUWACHSSCHWANKUNGEN. Schweiz. Ztschr. Forstw. 84: 261–272, 369–380, illus.

(9) ——— and BRÜCKMANN, W.
1930. HOLZZUWACHS UND WITTERUNG. Forstw. Centbl. 74: 380–403, illus.

(10) LODEWICK, J. E.
1930. EFFECT OF CERTAIN CLIMATIC FACTORS ON THE DIAMETER GROWTH OF LONGLEAF PINE IN WESTERN FLORIDA. Jour. Agr. Research 41: 349–363, illus.

(11) LORENZ, P.
1931. EIN BEITRAG ZUR METHODE SEINER BERECHNUNG UND SEINER AUSWERTUNG FÜR DIE UNTERSUCHUNG VON WIRTSCHAFTSKURVEN UND SONSTIGEN ZEITREIHEN. Berlin Inst. Konjunkturforschung, Vierteljahrshefte Konjunkturforschung Sonderheft 21, 104 pp., illus.

(12) MARSHALL, R.
1927. INFLUENCE OF PRECIPITATION CYCLES ON FORESTRY. Jour. Forestry 25: 415–429, illus.

(13) ROBBINS, W. J.
1921. PRECIPITATION AND THE GROWTH OF OAKS AT COLUMBIA, MISSOURI. Mo. Agr. Expt. Sta. Research Bull. 44, 21 pp., illus.

(14) SCHWEIZERISCHE METEOROLOGISCHE ZENTRALANSTALT.
1864–1926. ANNALEN DER SCHWEIZERISCHEN METEOROLOGISCHEN ZENTRALANSTALT. 1864–1926. Zurich.

(15) SNEDECOR, G. W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp., illus. Ames, Iowa.

THE INFLUENCE OF RAINFALL UPON TUFT AREA AND HEIGHT GROWTH OF THREE SEMIDESERT RANGE GRASSES IN SOUTHERN ARIZONA[1]

By PAUL B. LISTER, formerly *assistant forest ecologist, Southwestern Forest and Range Experiment Station,*[2] and FRANCIS X. SCHUMACHER, *senior silviculturist, Forest Service, United States Department of Agriculture*

INTRODUCTION

It is common observation that the forage stand of the semidesert grassland ranges of the Southwest varies greatly from year to year. While this variation is undoubtedly due to a number of factors, it is generally assumed to be governed predominantly by moisture made available through rain—an assumption that is scarcely to be questioned. Not all of the rainfall, however, can contribute effectively to growth of the forage species. An appreciable proportion of the water from showers may run off quickly in certain seasons of the year when rainfall is intense; or in other seasons, may evaporate directly from the soil in the face of dry winds or high temperatures; or the rain may be insufficient to saturate topsoils.

On the other hand, species or genera in the same plant association may not respond equally to the rainfall as the seasons progress; for it seems not unlikely that should one species or genus react to the same rainfall and in the same way as another species or genus with which it is associated, these two must be actively competing for moisture, and one of them—that one which is dominant in other physiological processes—may eventually suppress and finally supplant the other. But should one be particularly sensitive to, say, late winter and early spring rainfall while the other benefits largely from fall precipitation, they may, if other physiological processes are not decisive, grow together in apparent equilibrium. In short, differing degrees of response of the associated forage species to a unit of rain as the season advances may be a necessary condition of a climax, or even of a stable plant association.

It is the purpose of this paper to report the method and results of an investigation into the responses of three important forage grasses to variations in the amount and distribution of seasonal moisture supply on areas from which grazing animals had been excluded. These areas are on the Santa Rita experimental range of the Southwestern Forest and Range Experiment Station in southern Arizona.

THE DATA

The climate of the Santa Rita experimental range is characterized by long, hot summers with wide variations in temperature between day and night; short, mild winters; and low annual precipitation.

The lowest annual precipitation recorded at the range headquarters, 4,300 feet elevation, was 12.5 inches in 1903; the highest, 38.2 inches

[1] Received for publication Aug. 26, 1936; issued March 1937.

[2] Maintained by the U. S. Department of Agriculture at Tucson, Ariz., in cooperation with the University of Arizona.

in 1931; and the average from 1901 to 1931, 19.8 inches. Rainfall comes in two distinct periods, summer and winter. The summer period includes July to September, during which about 56 percent of the annual precipitation (July 1 to June 30) occurs; in the winter period from November to March about 36 percent occurs. The

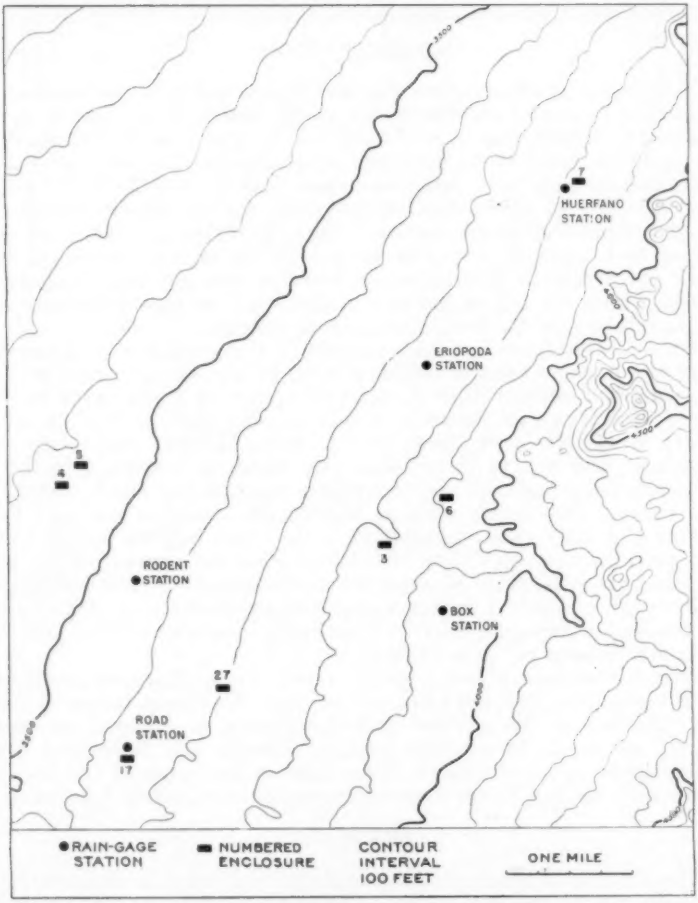

FIGURE 1.—Portion of the Santa Rita experimental range showing location of study plots and adjacent precipitation stations.

remaining 8 percent falls during the more or less dry periods of April, May, June, and October.

Before 1903 the area had been public domain open to uncontrolled grazing. In the early summer of 1903, however, the experimental range was fenced and thereafter largely protected from grazing animals until 1915, when it was again stocked with cattle.

Early work was devoted to observation of the natural restoration of the forage. In 1915, however, tests of the effect of various degrees of controlled grazing were begun. In order to provide check areas protected from grazing animals, five enclosures (nos. 3, 4, 5, 6, and 7 on fig. 1) were established in 1917 and two additional enclosures (nos. 17 and 27) in 1925.

Three precipitation stations, designated by the names of Box, Eriopoda, and Huerfano (fig. 1), were located in the vicinity of enclosures 3, 6, and 7, respectively; near enclosures 4 and 5 a precipitation station designated Rodent was established. Enclosures 17 and 27 were in the neighborhood of the already established Road precipitation station. Monthly precipitation was recorded at the stations, beginning in the summer of 1922.

Within the enclosures, on one or more sample quadrats each 1 square meter, the tuft areas of perennial grasses, 1 inch above ground, were mapped with a pantograph (*3*)[3] annually at the close of the growing season, and measurements were taken of the height of flower stalks of the perennial grasses within and around the quadrats. The quadrats within enclosures 4 and 5 were not, however, charted after 1929, and occasionally a charting was omitted at other enclosures.

The effect of rainfall distribution as reflected in yearly changes in tuft area and height growth was observed for the three principal grasses of the range, viz, three-awn grass (*Aristida* spp.),[4] black grama (*Bouteloua eriopoda*), and Rothrock grama (*B. rothrockii*). From the quadrat charts, the difference between tuft areas of the grasses in successive years (hereafter called density change) was computed in units of square centimeters for each quadrat. In a number of cases zero change in density occurred as a result of zero tuft area in each of 2 successive years. These were discarded from the study because they imposed undue limitation upon a freedom of variation of density change which would not have occurred if the quadrats had been large enough to preclude temporary extinction of the grasses thereon.

The density change and height-growth data are presented in table 1.

TABLE 1.—*Measurements of density change and height growth of flower stalks of three grasses according to enclosure and year of growth, Santa Rita experimental range*

DENSITY CHANGE[1]

Grass type and enclosure[2]	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932
	Square cm	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*
Three-awn:										
Enclosure 3 (1)	−239	39	−144						2	2
Enclosure 4 (1)	−9	8				−47	−1			
Enclosure 5 (1)	−3					−19	−21			
Enclosure 7 (1)	−66		2			−19	−5	25	47	68
Enclosure 17 (1)				44	94	−26	116	65	27	68
Enclosure 17 (2)				75	132	−151	−54	11	16	−4
Enclosure 17 (3)				24	20	−18	11	9	13	−10
Enclosure 17 (4)				31	46	−7	51	32	81	26
Enclosure 27 (2)				6	6	−2	0	29	−28	−2
Black grama:										
Enclosure 3 (1)	−9	3	−3							3
Enclosure 4 (1)	−34	15				−105	−14			
Enclosure 5 (1)	36	10				−75	−22			
Enclosure 6	−18	13	36			−48	−8	101	147	−7

[1] Averages for all quadrats and years are: Three-awn 6.5; black grama 28.3; Rockrock grama −4.5.
[2] Quadrat numbers given in parentheses.
[3] Reference is made by number (italic) to Literature Cited, p. 121.
[4] Mainly *Aristida divaricata*, but including small amounts, never exceeding 10 percent, of *A. orcuttiana*, *A. ternipes*, and *A. californica*.

TABLE 1.—*Measurements of density change and height growth of flower stalks of three grasses according to enclosure and year of growth, Santa Rita experimental range*—Continued

DENSITY CHANGE—Continued

Grass type and enclosure	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932
	Square cm	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*	*Square cm*
Black grama—Con.										
Enclosure 17 (1)				−6	66	−21	56	8	66	−66
Enclosure 17 (2)				33	107	−1	111	38	−23	−9
Enclosure 17 (3)				−3	73	−33	98	−30	48	54
Enclosure 27 (1)				31	235	−57	116	−3	98	122
Enclosure 27 (2)				35	105	−44	107	108	49	69
Rothrock grama:										
Enclosure 3 (1)	0	−11	20			−171	99	306	49	−60
Enclosure 4 (1)	−64	85				−66	−51			
Enclosure 5 (1)	−31	−30				−86	−132			
Enclosure 6	29	−55	−42			−140	64	223	−70	−114
Enclosure 7 (1)	109	−256	95			98	−62	85	−170	38
Enclosure 17 (1)				100	83	−229	−4	−5	−6	2
Enclosure 17 (2)				22	−23	−87	−4	2	−1	−7
Enclosure 17 (3)				−11	78	−89	17	68	−94	9
Enclosure 17 (4)				119	194	−381	37	85	−3	−62
Enclosure 27 (1)				−24	138	−184	243	15	−204	23
Enclosure 27 (2)				38	176	−294	251	−48	−46	24

HEIGHT GROWTH [3]

	Cm	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*	*Cm*
Three-awn:										
Enclosure 3 (1)	60	50	50		75		45		85	72
Enclosure 4 (1)	70	35			70	41	70			
Enclosure 5 (1)	65				45	36	45			
Enclosure 6	70	50			50	68	45	62	85	85
Enclosure 7 (1)	65	50	75		75	50	50	65	85	85
Enclosure 17 (1)			45		60	40	55	45	70	70
Enclosure 17 (2)			45		60	40	55	45	70	70
Enclosure 17 (3)			45		60	50	60	45	85	76
Enclosure 17 (4)			45		60	50	60	45	85	76
Enclosure 27 (1)				35		26	60	46	80	75
Enclosure 27 (2)				35		26	60	46	80	75
Black grama:										
Enclosure 3 (1)	55	25	45		60	42	40	52	60	60
Enclosure 4 (1)	65	35			50	46	50			
Enclosure 5 (1)	60	20			42	41	42			
Enclosure 6	65	30			55	42	40	41	50	50
Enclosure 7 (1)	60		50		60	48	50	54	53	52
Enclosure 17 (1)			45		55	40	50	50	63	60
Enclosure 17 (2)			45		55	40	50	50	63	60
Enclosure 17 (3)			45		45	35	50	50	65	60
Enclosure 17 (4)			45		45	35	50	50	65	60
Enclosure 27 (1)			50	45	50	35	45	48	58	56
Enclosure 27 (2)			50	45	50	35	45	48	58	56
Rothrock grama:										
Enclosure 3 (1)	50	35	40		50	36	35	45	60	50
Enclosure 4 (1)	60	30			40	39	40			
Enclosure 5 (1)	55	30			40	21	40			
Enclosure 6	55	35			50	38	35	40	53	50
Enclosure 7 (1)	55		60		57	42	40	50	58	52
Enclosure 17 (1)			45		45	35	35	45	58	52
Enclosure 17 (2)			45		45	35	35	45	58	52
Enclosure 17 (3)			45		45	35	25	40	60	50
Enclosure 17 (4)			45		45	35	25	40	60	50
Enclosure 27 (1)			40	40	50	36	45	40	58	54
Enclosure 27 (2)			40	40	50	36	45	40	58	54

[3] Averages for all quadrats and years are: Three-awn 58.7; black grama 49.2. Rockrock grama 44.6.

METHOD OF ANALYSIS

The method of analysis was first suggested by the researches of Fisher (*2*) concerning the influence of precipitation on the yields of wheat in England. Tippett (*8*) used the same method in an investigation of the effect of sunshine on wheat yields. It was used, also, in a recent analysis by Schumacher and Meyer (*6*) of data gathered by Dr. Hermann Knuchel in Switzerland relating to the influence

of precipitation on the growth of *Abies alba*. In brief outline, and taking density change of grass for illustration, the method is as follows:

The average effect of an additional inch of rainfall above the mean during a time interval (t) within a given period of time on density change (d) of a grass between one fall and the next may be expressed by the equation

$$d=\alpha_0+\alpha_1X_1(t)+\alpha_2X_2(t)+\text{------}+\alpha_kX_k(t) \tag{1}$$

in which $X_1(t)$, $X_2(t)$,------$X_k(t)$ are orthogonal functions of the time interval (t) during the period, and α_0, α_1, α_2,------α_k, are constants to be determined.

If the rainfall (r) within the same time interval (t) of the same period may be expressed

$$r=a_0+a_1X_1(t)+a_2X_2(t)+\text{------}+a_kX_k(t) \tag{2}$$

then the whole effect of the rainfall distribution during the period on change of grass density (D) from one fall to the next may be written

$$D-M_D=\sum_{t=-\frac{T}{2}}^{t=\frac{T}{2}}dr=\sum_{t=-\frac{T}{2}}^{t=\frac{T}{2}}(\alpha_0+\alpha_1X_1(t)+\alpha_2X_2(t)+\text{------}$$
$$+\alpha_kX_k(t))(a_0+a_1X_1(t)+a_2X_2(t)+\text{------}+a_kX_k(t)) \tag{3}$$

where M_D is the mean value of the observed annual changes in grass density. The summation is carried over all time intervals of the period, the first and last of which are denoted by $-\frac{T}{2}$ and $+\frac{T}{2}$, respectively, T being the number of time intervals within the period. On account of certain properties of the orthogonal functions $X_i(t)[i=0, 1, 2,\text{------}k]$, such as $\Sigma X_i=0$; $\frac{1}{T}\Sigma X^2{}_i=1$; and $\Sigma X_iX_j=0$ where $i\neq j$, $[j=0, 1, 2,\text{------}k]$, equation (3) becomes

$$D-M_D=T(\alpha_0a_0+\alpha_1a_1+\alpha_2a_2+\text{------}+\alpha_ka_k) \tag{4}$$

The object of the analysis is the pictorial representation of equation (1). The coefficients a_0, a_1, a_2,------a_k of equation (2) are first determined by the method of least squares from the rainfall record of each period at the precipitation stations. As many observation equations of the form of equation (4) are then set up as there are values of annual density change to be paired with the rainfall coefficients. Normal equations are derived from the observation equations according to the method of least squares and their solution affords estimates of the unknowns α_0, α_1, α_2,------α_k. Finally, when these are put in equation (1) the average effect appears of an additional inch of rain in each time interval on the annual change in grass density.

Period lengths of 15 months were chosen, beginning with June 1 of the year preceding growth and ending with August 31 of the

current year of growth. While September rainfall of the current year may also play a part in grass growth, it happened that during 2 years the quadrats were charted in September before the last of the summer rains. The effect of September rain on the current year's growth might not, as a consequence, be adequately evaluated, and it was therefore dropped from consideration.

The extension of the initial limit of the period beyond September was suggested by the work of Nelson (*5*), who pointed out that, in black grama range in southern New Mexico, the rainfall of the preceding summer effects significant changes in plant area from one fall to the next. He concluded that the value of the rainfall during the preceding summer was carried over into the current year's growth through the vigor imparted to the growing plant. Accordingly, to include the relatively high summer precipitation following comparatively dry spring weather, June 1 was chosen as the initial limit of the period. With the rainfall record by months, the time interval t is 1 month, and T, the number of intervals in the period, is 15.

THE RAINFALL DISTRIBUTION CONSTANTS

As stated above, the first part of the work is the determination of the coefficients a_0, a_1, a_2,------a_k of the orthogonal polynomial equation (2) for each period for which data are at hand. An immediate consideration is the degree of polynomial needed in order to describe adequately the rainfall distribution in each of the periods. Fisher (*2*), using the rainfall record at Rothamsted, England, calculated coefficients up to degree 5, but his data were according to intervals of 6 days in a period length of 1 year. Schumacher and Meyer (*6*) used sixth-degree polynomials to describe the precipitation distribution at Aarau, Switzerland, for time intervals of 1 month in a period of 15 months. Precipitation in the semidesert ranges of the Southwest is, however, relatively much more variable than in localities of greater abundance of rain. In view of this variability and the relatively long time interval employed, polynomials of degree 8 were decided upon.

The computational work, though tedious, is not complex; for if there are equal intervals of the independent variable, and if to every value of the independent variable there corresponds only one value of the dependent variable—as rainfall per month—all the computations necessary to calculate the curve representing the trend of (r) with respect to (t) for each period may be decidedly simplified through the use of orthogonal functions, as these have been tabulated by different authors. A very convenient set of tables is given by Lorenz (*4*), together with a full discussion of the theory of orthogonal functions and their application to specific problems.

Following the computational scheme and checking method as recommended by Lorenz—known as the scheme of Chetverikoff—the rainfall coefficients a_0, a_1, a_2,------a_8, of equation (2) were calculated from the rainfall record of each period at each station. These are listed in table 2 for units of 0.01 inch of rain per month. The rainfall trend for the average of the periods and stations as derived from the arithmetic average of each coefficient, is compared with the recorded average rainfall in figure 2.

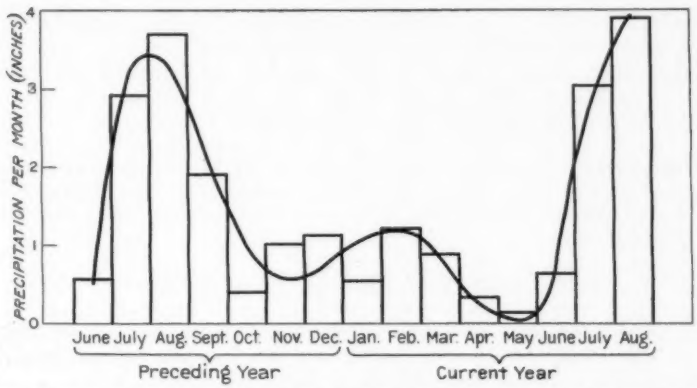

FIGURE 2.—Comparison of curve of rainfall trend (derived from arithmetic average of each coefficient) with recorded average monthly rainfall.

TABLE 2.—*Coefficients of orthogonal polynomials which express precipitation trends (in hundredths of inches per month) from June of a preceding year to August inclusive of a current year, according to precipitation stations; Santa Rita experimental range*

Precipitation station	Current year	Rainfall coefficients [1]								
		a_0	a_1	a_2	c_3	a_4	a_5	c_6	a_7	a_8
Box	1923	124	25	97	67	−35	16	−70	−53	−40
	1924	153	−39	35	35	−34	47	−89	31	−82
	1925	89	24	84	49	−2	42	−36	25	−21
	1927	126	16	48	85	29	52	−1	3	−5
	1928	122	−14	42	80	−12	53	−31	−25	45
	1929	116	18	51	62	7	58	−31	−17	3
	1930	155	−4	32	37	−56	48	−31	−50	41
	1931	207	107	156	126	112	75	5	48	−22
	1932	248	−52	116	109	−54	69	−73	−99	76
Rodent	1923	151	35	121	80	−36	48	−85	−59	−23
	1924	152	−64	41	35	−50	75	−86	35	−68
	1927	121	−15	24	71	−14	36	−8	−25	4
	1928	102	−9	30	56	3	29	−34	−20	19
	1929	95	−2	28	41	−11	39	−30	−21	1
Eriopoda	1923	132	5	98	67	−41	53	−81	−25	−37
	1924	148	−23	37	34	−44	23	−100	−1	−79
	1925	100	4	77	43	−2	39	−56	51	−33
	1927	128	14	50	90	18	50	14	−5	−15
	1928	119	−23	35	69	−32	40	−42	−50	36
	1929	115	15	54	65	12	56	−29	−8	−15
	1930	171	26	50	46	−63	34	−59	−71	28
	1931	204	63	164	107	89	92	−27	57	−5
	1932	207	−45	100	91	−63	52	−93	−94	44
Huerfano	1923	142	11	114	81	−24	60	−69	−20	−32
	1924	161	−25	37	44	−42	35	−95	0	−70
	1925	100	16	89	49	5	53	−53	54	−23
	1927	158	−10	48	89	−1	60	−11	1	−24
	1928	142	−37	36	90	−40	46	−19	−58	48
	1929	125	14	51	67	−4	46	−37	−36	−13
	1930	170	18	53	47	−45	58	−51	−37	29
	1931	217	73	157	116	94	85	−10	39	39
	1932	230	−60	95	90	−65	40	−88	−115	49
Road	1925	87	24	58	37	−12	14	−34	14	−5
	1926	148	−20	37	39	−3	26	−7	−32	−23
	1927	140	−13	40	82	−16	50	−10	−29	9
	1928	122	−8	49	75	−1	45	−33	−26	25
	1929	129	9	47	52	−31	36	−49	−39	5
	1930	181	−2	48	17	−49	42	−27	−40	33
	1931	210	89	173	99	103	58	−5	13	14
	1932	230	−64	106	102	−54	82	−78	−63	52

[1] Average a_0, 149.425, a_1, 1.925, a_2, 70.200, a_3, 68.025, a_4, −11.600, a_5, 49.050, a_6, −43.725, a_7, −18.675, a_8, −1.000.

REGRESSION OF ANNUAL DENSITY CHANGE AND HEIGHT GROWTH ON RAINFALL IN DIFFERENT SEASONS

The unknowns α_0, α_1, α_2, ... α_8 of equation (4) were next calculated by the method of least squares (*1, ch. XIV*) from the data of tables 1 and 2, after setting up observation equations of the dependent variable (density change or height growth of one of the grasses, as the case might be) in terms of the rainfall coefficients a_0, a_1, a_2, ... a_8 for corresponding station and year. As an illustration, the observation equation for change of density of three-awn grass between the falls of 1922 and 1923 at Box station is

$$-245.5=124T\alpha_0+25T\alpha_1+ \ldots\ldots -40T\alpha_8+C$$

where C is a constant, also to be determined. Each set of observation equations leads to the normal equations which, when solved simultaneously, render 15 (since $T=15$) times the coefficients α_0, α_1, α_2, ... α_8 of equation (4) and the constant C. Their numerical values are listed in table 3 according to the density change or height-growth equation to which they belong for each of the three grasses.

TABLE 3.—*Regression coefficients and constant terms of equations which express calculated departures of density change or height growth of flower stalks of 3 range grasses from their respective averages, in terms of the rainfall coefficients of the 15-month periods*

DENSITY CHANGE

Species	Regression coefficients									Constant term
	$15\alpha_1$	$15\alpha_2$	$15\alpha_3$	$15\alpha_4$	$15\alpha_5$	$15\alpha_6$	$15\alpha_7$	$15\alpha_8$	$15\alpha_9$	
Three-awn	1.1349	0.4985	−1.5968	−0.2768	0.7292	1.8153	0.0467	−0.7048	−0.4693	−139.7243
Black grama	1.4772	2.0174	−1.3738	2.6970	−2.5859	−.2139	1.5715	1.5045	−.6613	−217.3320
Rothrock grama	.4456	1.5617	.6979	.7796	−4.2941	1.7472	4.0215	.1654	−1.9016	−125.4921

HEIGHT GROWTH

Three-awn	0.1284	0.2078	0.0404	0.3803	−0.2269	−0.0378	−0.0226	0.1102	0.0028	−47.7886
Black grama	.0156	.0964	.2639	.1367	−.3251	.0124	.3295	−.0258	−.0854	−21.6410
Rothrock grama	.0147	.0385	.2489	.1490	−.2654	−.0533	.2442	.0672	−.0639	−18.7090

Tests of significance are based upon analyses of variance (7) as presented in table 4. In general

$$\Sigma(y-M_y)^2=\Sigma(y-Y)^2+\Sigma(Y-M_y)^2$$

in which y is an observed value of the dependent variable, the arithmetic average of which is M_y, and Y is the estimate of y by the method of least squares. The left-hand member of the above identity is the total sum of squares of density change or height growth before eliminating that part due to their regression on rainfall distribution during the 15-month periods. The sum of squares due to regression is calculated through the identity

$$\Sigma(Y-M_y)^2=T\alpha_0\Sigma(a_0y)+T\alpha_1\Sigma(a_1y)+T\alpha_2\Sigma(a_2y)+\ldots\ldots+T\alpha_8\Sigma(a_8y)$$

for which the sum of products of each term of the right-hand member was computed previously as steps leading to the normal equations.

The remaining sum of squares, $\Sigma(y-Y)^2$, is due to deviation from regression, that is, it is that part of the total sum of squares that has been found to be independent of the rainfall distribution.

TABLE 4.—*Analyses of variance of density changes and height growth*

DENSITY CHANGES

Range grass and source of variation	Sum of squares	Degrees of freedom	Mean squares	*F* observed	*F* where *P*=0.01
Three-awn:					
Regression	71,241	9	7,916	2.85	2.85
Deviation from regression	122,339	44	2,780		
Total	193,580	53			
Black grama:					
Regression	103,318	9	11,480	4.42	2.84
Deviation from regression	116,852	45	2,597		
Total	220,170	54			
Rothrock grama:					
Regression	397,988	9	44,221	4.19	2.70
Deviation from regression	676,255	64	10,566		
Total	1,074,243	73			

HEIGHT GROWTH

Three-awn:					
Regression	11,664	9	1,296.0	13.43	2.71
Deviation from regression	6,077	63	96.5		
Total	17,741	72			
Black grama:					
Regression	6,368	9	707.6	20.16	2.68
Deviation from regression	2,420	69	35.1		
Total	8,788	78			
Rothrock grama:					
Regression	4,878	9	542.0	22.21	2.68
Deviation from regression	1,683	69	24.4		
Total	6,561	78			

Sums of squares due to these three sources of variation are listed in the second column of table 4. In the third column are the degrees of freedom—one less than the numbers of observations for total, one for each independent variable (a_0, a_1, a_2, ---- a_8) for the regression, and the remaining number for departures from regression. In the fourth column are the mean squares.

If density change or height growth of the grasses were independent of the rainfall distribution, the mean square due to regression would be approximately equal to the mean square of the departures from regression, and the ratio of the former to the latter—designated *F*—would therefore be 1, approximately. Under such conditions, values taken from the distribution of *F* in random samples (*7, pp. 88–91*) as given in the last column would be exceeded but once in 100 sets. By comparison, the observed values of *F* in the next to last column are as great or greater. All the correlations of grass growth with the rainfall distribution are consequently adjudged highly significant.

The issue, however, is not the obvious truth that rainfall influences grass growth; but since the data at hand are sufficient to corroborate

what is already known, the issue is, rather, to indicate the extent of rainfall influence at different seasons of the period.

Putting, therefore, the set of coefficients α_0, α_1, α_2, ---- α_8, which express annual change in density, or height growth of one of the grasses concerned, into equation (1), where

$$\dot{d}=\alpha_0+\alpha_1X_1(t)+\alpha_2X_2(t)+\text{----}+\alpha_8X_8(t),$$

the functions $X_1(t)$, $X_2(t)$, ---- $X_8(t)$ being taken from Lorenz's

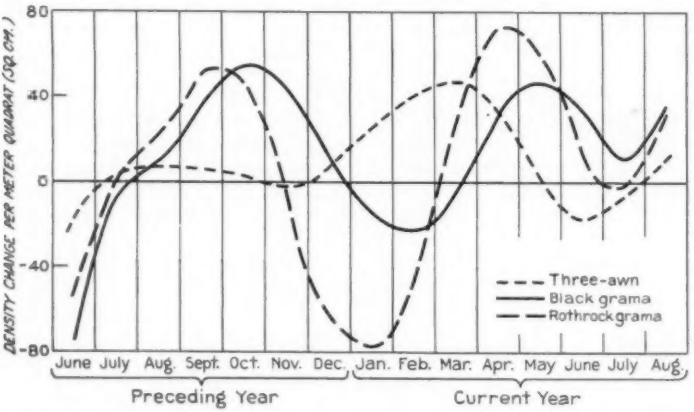

FIGURE 3.—Computed effect of an additional inch of rain per month on annual change in density of three grasses.

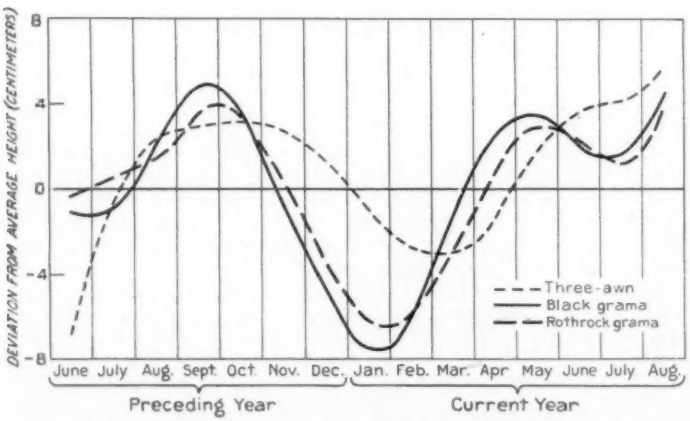

FIGURE 4.—Computed effect on yearly growth of flower stalks of an inch more of rain per month.

tables (*4*),[5] and plotting on t, we have the graphic expression of the effect of an additional one-hundredth inch of rain per month, according to the months of the period, on annual change in density or height

[5] Lorenz's tables do not give the functions $X_i(t)$ for i greater than 6. Consequently $X_7(t)$ and $X_8(t)$ were calculated for each of the 15 values of t through the use of equation (11) of Lorenz's paper (*4*, p. 31).

growth. The results (multiplied by 100) are presented graphically in figure 3 to show the average effect of 1 additional inch of rain per month on annual density change in square centimeters per meter quadrat; and in figure 4, on height growth of flower stalks in centimeters per year. The ordinates of these figures represent deviations from the mean values of density change or height growth listed at the bottom of table 1.

DISCUSSION

The curves of figures 3 and 4 represent the average benefit to the forage stand, in tuft area and height growth, of 1 inch of rain per month during the 15-month period in excess of each monthly rainfall average as given by the distribution curve of figure 2. Change in sign of the ordinate scales would, of course, represent the corresponding average damage chargeable to 1 inch of rain per month in defect of its monthly average.[6] Likewise the best estimate of density change or height growth as a result of no change from the monthly averages—i. e., the average rainfall distribution itself—would be zero deviation from the averages given at the bottom of table 1—i. e., the general averages themselves for all stations and years.

It is the experience of range workers that the three grasses under discussion associate in apparent tolerance of one another. The trends of figure 3 suggest that this may be due to differences in response of the grasses to rainfall distribution. The first general impression conveyed by the figure is that rainfall does not affect the density changes of the three grasses alike, except at seasons—late July of the preceding and current years—when the effect of rainfall variation is rather negligible.

Closer inspection of figure 3 reveals that the curves of the grama grasses show approximately the same trends, the differences between them being that black grama lags by about a month behind Rothrock grama and is, moreover, less sensitive to rainfall variation of the winter and spring.

The average tuft area per meter quadrat during the years of observation was 168 cm^2 of black grama, 162 cm^2 of Rothrock grama, and 76 cm^2 of three-awn. For reasons that are not clear, additional rainfall during June of the preceding year results in loss to the current year's density growth of black grama at an average rate of 76 cm^2 per inch (45 percent of average tuft area); of Rothrock grama at an average rate of 53 cm^2 per inch (33 percent). Although variation of July precipitation of the preceding year has little effect on density change of either of the gramas, additional rain during the late summer benefits both. Maximum benefits, coming from the late September excess to Rothrock grama and from the late October excess to black grama, reach 56 cm^2 per inch or somewhat over 30 percent.

Additional rainfall during the winter season, on the other hand, has a deleterious influence on the density growth of the gramas, particularly on that of Rothrock grama, which is subject to an average loss of 80 cm^2 (49 percent) per inch of additional January rain. The greatest loss to black grama density is 26 cm^2 (15 percent) per inch of additional February rain.

Extra spring precipitation is beneficial to the gramas, its maximum effect reaching 70 cm^2 (42 percent) per inch of April rain to Rothrock

[6] In this case it is recognized, of course, that as much as an inch in defect of most of the monthly averages from October to June is impossible

grama and 44 cm² (27 percent) per inch of May rain to black grama. Thereafter the benefit is lessened, and in middle summer it is negligible. August excess, however, again benefits the density growth of the gramas to an extent of about 30 cm² (18 percent) per additional inch.

The reaction of three-awn tuft area to precipitation is quite distinct from that of the grama grasses. Except during June and early July, additional precipitation during the preceding summer and fall has little effect on its density. An average density loss of 20 cm² (26 percent) is associated with an additional inch of June precipitation in both the preceding and current years.

During the winter season, additional rain becomes progressively more beneficial to the tuft area of three-awn, reaching its maximum in March with 45 cm² (59 percent) per inch. Additional April precipitation is less beneficial, that of May ineffective, and that of June, again, depressive. Thereafter tuft area of three-awn is but negligibly affected by variation in rainfall.

Turning now to figure 4, it is at once apparent that additional rain coming during the preceding fall and current spring and summer benefits the height growth of the three grasses during the summer of the "current year", but coming during the winter it mostly detracts from it. The practically identical effect of the rainfall distribution on the height growth of the two gramas, in contrast to its singular influence on three-awn, is also striking.

During the years of observation the average height of the flower stalks of black grama was 49 cm; of Rothrock grama, 45 cm; and of three-awn, 59 cm.

Until late summer of the preceding year, additional rainfall has little influence on the current year's height growth of the grama flower stalks. But with the advent of August the efficiency of additional rainfall mounts, reaching a maximum benefit in late September of 5 cm per inch for black grama and 4 cm per inch for Rothrock grama, or 9 to 10 percent of the average height in either case. As fall advances, the benefit lessens, dropping through zero in November to a loss as a result of excess winter precipitation, which reaches 7.5 cm per inch for black grama and 6.4 cm per inch for Rothrock grama (14 to 15 percent) in January. Additional spring rainfall is again beneficial, particularly in May, when the benefit reaches 3.5 cm per inch for black grama and 3 cm per inch for Rothrock grama (7 percent). Thereafter the benefit is lessened to a minimum of about 15 cm per inch in July before increasing again with advancing summer. This same effect of July rain on density change is apparent in figure 3. A not unlikely reason for this seeming loss of rainfall efficiency is that with the bursting of flower buds the benefit of additional rain is withdrawn from height and tuft area growth and is put into flowers and seed.

Roughly, the rainfall distribution affects height growth and density change of the two grama grasses alike, for the curves of figures 3 and 4 show generally the same trends. In three-awn, on the other hand, height of flower stalks and density change are affected quite differently by additional rain. For whereas additional rain during the preceding summer and fall scarcely affects density change at all, it contributes at an average rate, from August to the end of the preceding year, of 2.5 cm (4 percent) per inch per month to height growth during the

summer of the current year. During the later winter, when additional rain greatly benefits tuft area, it detracts from height growth at a rate that reaches 3 cm (5 percent) per inch of additional March rain. By June the influence of rain is again reversed, an additional inch effecting a loss of 26 percent to density but a benefit of about 3.5 cm (6 percent) to height growth. Through July and August this benefit is somewhat increased.

SUMMARY

The effect of variation in precipitation distribution during a 15-month period on the annual density change and height growth of the three forage grasses—three-awn grass (*Aristida* spp.), black grama (*Bouteloua eriopoda*), and Rothrock grama (*B. rothrockii*)—within the growing season of the current year, was determined by statistical analysis. The method used consisted in describing the observed monthly precipitation during each 15-month period, ending with August for each station and year, by orthogonal polynomials of degree 8; the coefficients of these were then used as independent variables in six multiple-correlation analyses, the dependent variables of which were (1) the annual density change and (2) the height growth of flower stalks, of each of the three grasses according to station and year. All the correlations are highly significant.

Graphic expressions of the average effect of an added inch of precipitation per month indicate, in the case of the two grama grasses, that the most beneficial precipitation distribution from normal for both density change and height growth consists of relatively dry winters sandwiched between relatively wet autumns and springs. Three-awn grass seems admirably adapted to the variation in precipitation distribution characteristic of the area studied in that either positive or negative departure from normal seasonal precipitation benefits, in general, either density change or height growth, although not both at once.

LITERATURE CITED

(1) Bruce, D., and Schumacher, F. X.
1935. FOREST MENSURATION. 360 pp. New York and London.

(2) Fisher, R. A.
1925. THE INFLUENCE OF RAINFALL ON THE YIELD OF WHEAT AT ROTHAMSTED. Roy. Soc. [London], Phil. Trans., Ser. B, 213: 89–142, illus.

(3) Hill, R. R.
1920. CHARTING QUADRATS WITH A PANTOGRAPH. Ecology 1: 270–273, illus.

(4) Lorenz, P.
1931. EIN BEITRAG ZUR METHODE SEINER BERECHNUNG UND SEINER AUSWERTUNG FÜR DIE UNTERSUCHUNG VON WIRTSCHAFTSKURVEN UND SONSTIGEN ZEITREIHEN. Berlin Inst. Konjunkturforschung, Vierteljahrshefte Konjunkturforschung, Sonderheft 21, 104 pp., illus.

(5) Nelson, E. W.
1934. THE INFLUENCE OF PRECIPITATION AND GRAZING UPON BLACK GRAMA GRASS RANGE. U. S. Dept. Agr. Tech. Bull. 409, 32 pp., illus.

(6) Schumacher, F. X., and Meyer, H. A.
1936. EFFECT OF CLIMATE ON TIMBER-GROWTH FLUCTUATIONS. Jour. Agr. Research 54: 79–107, illus.

(7) Snedecor, S. W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp., illus. Ames, Iowa.

(8) Tippett, L. H. C.
1926. ON THE EFFECT OF SUNSHINE ON WHEAT YIELD AT ROTHAMSTED. Jour. Agr. Sci. [England] 16: [159]–165, illus.

PRACTICAL FIELD METHODS OF SAMPLING SOIL FOR WIREWORMS [1]

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INTRODUCTION

To be able to estimate accurately the number of wireworms in any given field is a fundamental necessity if progress is to be made in economic investigations of these insects. With valid methods for estimating the size of wireworm infestations in the soil the following important objectives are attainable: (1) The relative effectiveness of various practices for controlling wireworms can be determined by statistical treatment; (2) the actual degree of crop damage inflicted by different numbers of wireworms can be measured; (3) knowledge of the changes in degree of infestation in the field, annually or over longer periods, in conjunction with a study of soil factors, will help toward the discovery and measurement of the factors affecting wireworm incidence.

None of the papers on quantitative wireworm investigations thus far published refer to the degree of accuracy obtained by the sampling method used. A review of the literature indicates that sampling methods for censuses of wireworm populations should be studied to determine which method gives the most accurate results.

In studying wireworm populations several methods of sampling were tried. At first the samples taken [3] were large, the units being 1 square yard or more in surface area. These large units were too few in number, and the labor involved in their examination proved excessive. Efforts were therefore directed toward developing a time- and labor-saving system that would give fairly accurate results. Smaller sampling units, of 1 square foot and less, were studied and will be reported upon in this paper.

REVIEW OF LITERATURE

Several British investigators refer to their soil sampling of unit blocks 9 inches square on the surface and 9 to 12 inches deep. Thus, Roebuck (*9*)[4] dug out portions of soil in different parts of a field, carefully crumbled and sifted it, and counted the wireworms. The size of his sampling unit was at first a 1-foot cube, but later a block 9 inches square on the surface and 1 foot in depth was used. The number of units in a field ranged from 5 to 12, and in subsequent counts on the same field the same number of units were taken. The average number of wireworms found in the unit samples from a field was then multiplied by a conversion factor to obtain an estimate of the number

[1] Received for publication June 18, 1936; issued March 1937.

[2] The work reported in this paper was done under the direction of M. C. Lane, entomologist, in charge of Pacific Northwest wireworm investigations, Walla Walla, Wash. M. C. Lane, F. H. Shirck, R. S. Lehman, and K. E. Gibson assisted in the accumulation of the data.

[3] Wireworm-population studies were begun in 1926 by M. C. Lane and F. H. Shirck at Toppenish, Wash.

[4] Reference is made by number (italic) to Literature Cited, p. 134.

per acre. The sampling units of both Morris (*7*) and Edwards (*1*) were 9 inches square on the surface and 9 inches deep. In his faunal studies of arable lands at Rothamsted, Morris examined as many as 23 sampling units from each half-acre plot used, and states that they were never taken nearer together than about a yard. The unit samples were carried to the laboratory and, after being dried on brown paper, the insects were counted in a small quantity of soil at a time. Edwards examined an average of four units per plot in his insect surveys. In sampling for wireworms King and Glen (*4*) and Wardle (*11*) both used units 1 square foot in area, King and Glen taking 10 units per plot.

In an earlier publication King (*3*) refers to the use of a smaller unit, 0.1 square foot, but he does not say how many of these units were taken. For preliminary investigations Hawkins (*2*) used units 1 square foot in surface area, extending to a depth of 6 to 8 inches, but he does not state how many units were taken. Lacroix (*5*) determined the size of larval populations by excavating two areas, each 2 feet square, to a depth of 2 feet.

METHODS AND PROCEDURE

APPARATUS AND EQUIPMENT

The difficulty of sampling is increased because, since the wireworms live below the surface of the ground, there is no indication of their whereabouts. As there is no natural unit of measurement, arbitrary units had to be adopted. In this study the standard sampling unit comprises the soil under an area 1 square foot in size, the depth ranging from 1 to 2 feet according to the season. The unit will therefore be designated by area, it being understood that the depth chosen was considered sufficient for obtaining all wireworms. The 1-square-foot unit is large enough to make it certain that wireworms will be obtained in the majority of cases, and it is as small as can be used where depth records are desired for studies of biology and control.

For taking the samples a metal form of 10-gage black iron, 1 foot square and 8 inches high with a 1-inch flange at the top, was devised (fig. 1, *A*). This form is pushed into the ground and the soil removed in 3-inch layers with a square-edged shovel. Each layer approximately fills a 10-quart galvanized-iron pail, which makes an easy measure. The number of pails of soil per unit area depends on the depth of the sampling.

Two smaller sampling units were also investigated. One of these has a surface area of one-fourth square foot. A metal form of 8-gage black iron, 6 inches square at the bottom, 6¼ inches square at the top, and 12 inches high, was made with a 1-inch flange of 1-gage iron welded to the top edge (fig. 1, *B*). A tamper was made of a steel plate 8½ inches square and five-eighths of an inch thick, with a chromium-steel automobile drive shaft (1¼ inches by 4¼ feet) riveted loosely at one end through the center of the plate (fig. 1, *D*). The form is driven into the soil its entire depth with the tamper, and then the ¼-cubic-foot section of soil is removed intact by using the steel plate of the tamper under the flange as a pry. The soil is then shaken into a pail for sifting. The form, being slightly larger at the top, allows easy removal of the soil by shaking. Soil below a depth of 12 inches is removed with a soil auger (fig. 1, *E*).

The smallest sampling unit studied is one-sixteenth of a square foot. The same type of square metal form was used as with the larger units, the lower opening being 3 inches square and the top opening 3¼ inches square to allow easy removal of the soil (fig. 1, *C*). This size is the most convenient of the three to use, as the metal form is more easily driven into the soil and lighter to handle. The unit of soil is small enough to be run through a washing device (fig. 2) (*10*), whereby all stages of wireworms, even the eggs, can be almost completely recovered.

Very early in the investigations a mechanical soil sifter was developed to reduce labor and increase the number of sampling units possible to be taken in a given time. The first machine (*6*) devised was operated by hand. A more mobile power sifter [5] has finally been developed with sifting capacity of approximately 5 cubic feet per hour, although the

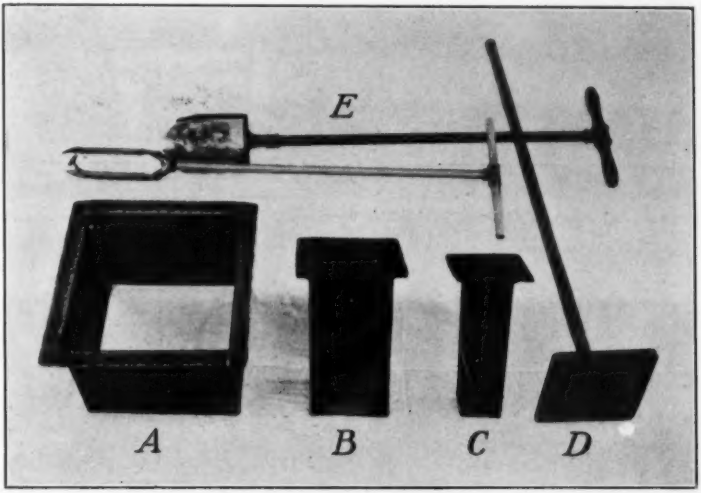

FIGURE 1.—Equipment and forms used in taking soil samples to determine wireworm populations: *A*, 1-square-foot sampler; *B*, ¼-square-foot sampler; *C*, 1/16-square-foot sampler; *D*, steel tamper; *E*, soil augers for use in obtaining samples deeper than first surface foot.

number varies according to the moisture content, texture, and compactness of the soil. This sifter in its final development is pictured in figure 3. For best results a screen of 4 meshes to the inch is used on top for breaking up lumps and removing trash. Under that a 10- or 12-mesh screen can be used to catch adults and large wireworms. The mesh of the bottom screen should be small enough to catch the smaller wireworms yet large enough to allow most of the soil to pass through. The usual size is 16 mesh, but it is safer to employ 18 mesh with dry soils. Experience has demonstrated that never more than 10 percent of the small wireworms (5–10 mm) pass through the sifter screens, the loss being less when the soil is wet. When it is desired to obtain census counts of eggs and very small or newly hatched larvae, the soil should be washed through screens of 40 to 60 mesh. The most

[5] LANE, M. C., and SHIRCK, F. H. A MOBILE POWER SOIL SIFTER. U. S. Dept. Agr., Bur. Ent. and Plant Quarantine Multigraphed Circ. ET–70, 2 pp., illus. 1936.

FIGURE 2.—Soil-washing device used in obtaining wireworm populations in ¼₀-square-foot units of soil.

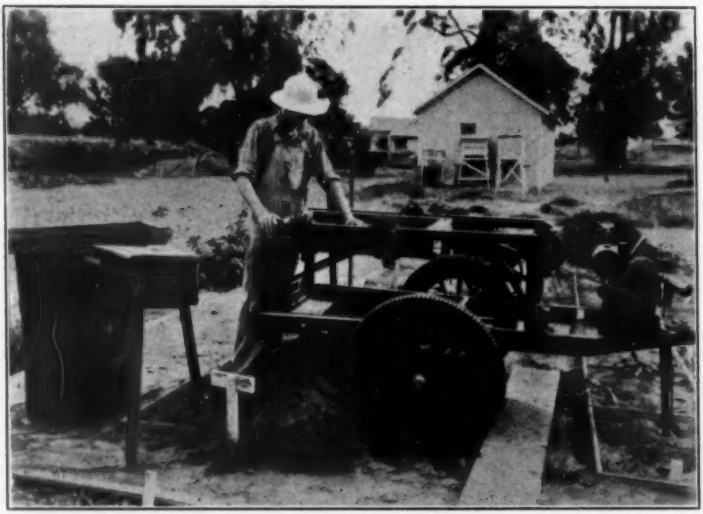

FIGURE 3.—Power soil sifter used in obtaining populations of wireworm adults and larvae.

accurate population counts that are obtained by soil sifting are taken during the spring months, before the current year's brood is hatched.

Only one pail of soil from 1 square foot of surface to a depth of 3 inches is placed on the sifter at one time. The numbers of wireworms found are recorded on standard forms (figs. 4 and 5) according to the depth, their stage of development, and the condition of the soil.

Form EQ-20 Date

Place Notes by

Sample No.	Soil Depth	Larvae	Pupae	Adults	Soil Depth	Larvae	Soil Data			Remarks
							Depth	Temp.	Moist.	
	0"- 3"				12"-15"					
	3"- 6"				15"-18"					
	6"- 9"				18"-21"					
	9"-12"				21"-24"					
	0"- 3"				12"-15"					
	3"- 6"				15"-18"					
	6"- 9"				18"-21"					
	9"-12"				21"-24"					
	0"- 3"				12"-15"					
	3"- 6"				15"-18"					
	6"- 9"				18"-21"					
	9"-12"				21"-24"					
	0"- 3"				12"-15"					
	3"- 6"				15"-18"					
	6"- 9"				18"-21"					
	9"-12"				21"-24"					
	0"- 3"				12"-15"					
	3"- 6"				15"-18"					
	6"- 9"				18"-21"					
	9"-12"				21"-24"					

FIGURE 4.—Form used for recording soil-sifting data.

SOIL MOISTURE DATA

Sample No.	Soil Depth	Can No.	Total Weight Wet	Total Weight Dry	Net Weight Dry	Weight of Water	Moisture Per Cent
	0"- 3"						
	3"- 6"						
	6"- 9"						
	9"-12"						
	12"-15"						
	15"-18"						
	18"-21"						
	21"-24"						

FIGURE 5.—Reverse of form shown in figure 4, used for recording soil-moisture data.

METHODS OF SELECTING UNIT AREAS

In selecting unit areas it is not possible, strictly speaking, to obtain a theoretically random sample, but the plan followed was to take representative unit areas in each field. Sampling was begun at one end of a field and continued across to the other end. The practice was to scatter the unit areas over the field as evenly as possible, each succeeding area being located by a toss of the metal form. This plan has proved the most feasible and comes nearest to giving a theoretically random sample of the wireworm population in a field.

Because of the difficulty in obtaining samples, it is of scientific value to know whether the soil in the smaller unit areas (one-fourth or one-sixteenth square foot) will give as good an estimate of wireworm density as the soil in the larger unit of 1 square foot. Again, it is

important to know whether a sample of 25- or 50-unit areas will be as good as a sample of 100-unit areas. The size of the unit area and the number of units necessary to constitute a sample are discussed in this paper.

During the past several years data on the wireworm populations of a large number of fields have been obtained for this statistical study, several thousand unit areas having been examined and the wireworm count recorded for each.

PRESENTATION OF DATA

DETERMINATION OF THE TYPE OF WIREWORM DISTRIBUTION

In the present problem the variation in the mean number of wireworms from a given number of sampling units would be given by the standard error of that mean. This statistic is valid provided the sampling units differ from a homogeneous population solely by errors of random sampling. It is necessary, therefore, that a test of the homogeneity of the wireworm distribution be made.

In table 1 are given the observed and the expected frequencies of a Poisson series (Pearson (*8*)) for unit areas of 1, ¼, and 1/16 square foot. The graphs representing these frequency distributions are given in figure 6. In applying the χ^2 test to such a series, in no class should the number expected be less than 5, since the calculated distribution of χ^2 is not closely realized for very small classes.

TABLE 1.—*Comparison of the observed and expected frequency distributions of wireworm-population samples obtained from 1-, ¼-, and 1/16-square-foot unit areas, each sample consisting of counts from 100 units*

Wireworms per unit area (number)	Frequency of occurrence in sampling units of—					
	1 square foot		¼ square foot		1/16 square foot	
	Observed	Expected	Observed	Expected	Observed	Expected
0			43	41	81	82
1	[1] 16	[1] 8	40	37	16	16
2	13	13	8	16	[2] 3	[2] 2
3	17	19	[2] 9	[2] 6		
4	14	19				
5	14	16				
6	9	11				
7	6	7				
8 and over	11	7				
Total	100	100	100	100	100	100
χ^2		12.47		5.84		.51
Degrees of freedom		6		2		1
Probability		.05		.05		.40

[1] 1 or 0 wireworm per unit area.
[2] 2 or more wireworms per unit area.
[3] 3 or more wireworms per unit area.

In testing the 1-square-foot unit the frequencies for 0 wireworm and 1 wireworm and also those for 8 or more wireworms are pooled. The comparison obtained is given in table 1. When eight frequency classes are used, $\chi^2=12.47$. In ascertaining the value of n, the expected frequencies are calculated, not only from the total number of values observed (100), but also from the observed mean. There

remain, therefore, 6 degrees of freedom, and $n=6$. The χ^2 table shows that for this value P is between 0.10 and 0.05, agreeing closely with expectation.

In like manner the smaller sampling units are tested. According to the χ^2 test for each of these surface areas, the actual wireworm

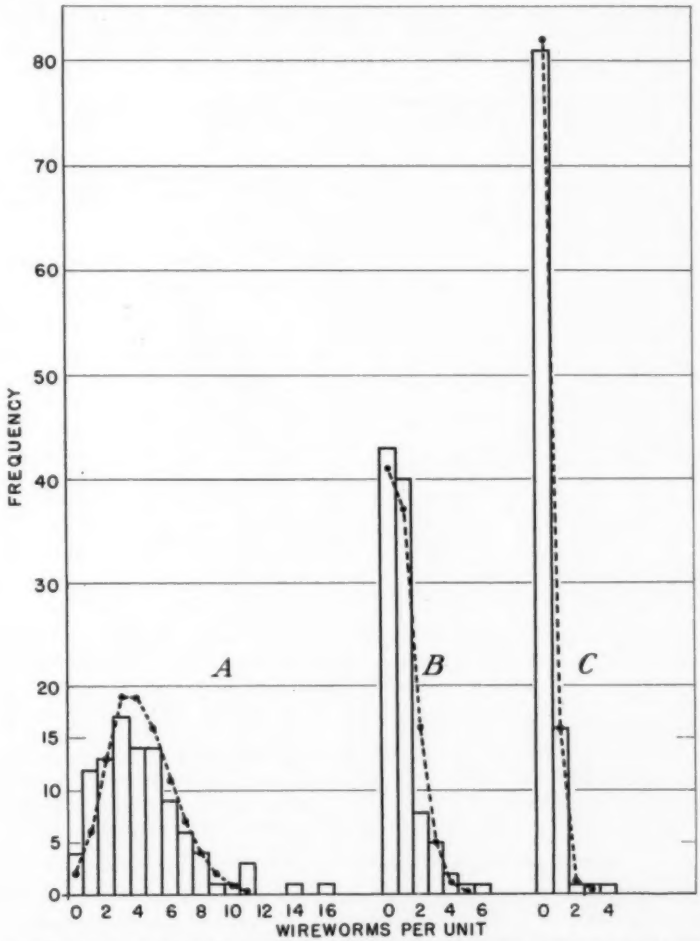

FIGURE 6.—Wireworm distribution per 100 units: *A*, 1-square-foot units; *B*, ¼-square-foot units; *C*, 1/16-square-foot units. Rectangular areas, observed distribution; curve, expected frequencies of Poisson series.

populations fall into Poisson series, indicating the uniformity of the population and the usefulness of the technique employed. In the case of the 1-square-foot sampling units, the distribution is moderately asymmetrical (fig. 6, *A*), the class frequency decreasing with greater rapidity on one side of the maximum than on the other.

Because of the preponderance of the zero-infestation units, the other two distributions obtained from the small surface areas represent reversed J-shaped curves (fig. 6, *B*, *C*).

COMPARISON OF SIZES OF SAMPLING UNITS

Tests were made in seven fields to compare the 1-square-foot, the ¼-square-foot, and the 1/16-square-foot unit areas. The population statistics obtained by the three methods of sampling in the same field are presented in table 2.

TABLE 2.—*Comparison of the mean wireworm populations in 1-square-foot, ¼-square-foot, and 1/16-square-foot unit areas obtained from the same field*

Field no.	Units in sample	Size of unit area	Average wireworms	Coefficient of variation	Relative standard error
	Number	*Square feet*	*Number* [1]	*Percent*	*Percent*
1	25	1	5.40±0.79	73	14.6
	25	¼	.96± .24	125	25.0
	25	1/16	.36± .11	152	30.6
2	25	1	5.44± .91	84	16.7
	25	¼	1.56± .27	86	17.3
	25	1/16	.80± .21	131	26.3
3	25	1	8.72±1.33	76	15.3
	25	¼	2.04± .50	122	24.5
	25	1/16	.64± .17	132	26.6
4	25	1	19.00±3.20	84	16.8
	25	¼	5.56±1.20	108	21.6
	25	1/16	1.52± .41	135	27.0
5	50	1	5.42± .59	77	10.9
	50	¼	1.26± .20	112	15.9
	50	1/16	.58± .12	151	20.7
6	50	1	13.55±1.80	95	13.3
	50	¼	3.73± .66	126	17.7
	50	1/16	1.10± .22	144	20.0
7	100	1	9.64±1.06	109	11.0
	100	¼	2.53± .38	150	15.0
	100	1/16	.83± .13	159	15.7

[1] The standard error and not the probable error is used after the ± sign.

In comparing samples consisting of the same number of units, i. e., 25, 50, or 100, it will be seen that the coefficient of variation for the 1-square-foot unit is consistently smaller than for the other two units. The relative standard error, which may be defined as the standard error expressed as a percentage of the mean, likewise is consistently smaller. By the consistency of results it is shown that the 1-square-foot unit gives a better estimate of the wireworm population than the smaller units. The ¼-square-foot samples are also shown to be more accurate than the 1/16-square-foot samples.

NUMBER OF UNITS NECESSARY TO CONSTITUTE A SAMPLE

One-square-foot, one-quarter-square-foot, and one-sixteenth-square-foot units were taken in bare fields and in crop-covered fields ranging in size from a small fraction of an acre to several acres. In table 3 are given the mean population levels for 25 fields, obtained by taking 25, 50, and 100 units of each size from the same field, together with their standard errors and the relative standard errors. For the smaller units the population means, naturally, are smaller. These figures can be placed on a comparable basis by multiplying by the proper conversion figure, 4 or 16. An explanation might be made at this point regarding the very large number of wireworms obtained in some of the fields by the

1/16-square-foot units. In these cases samples were taken in fields to determine the total population of newly hatched wireworms. The statistics in table 3 show that the relative standard error is not only smallest but most stable for the 100 units. The 50 units show good results also.

TABLE 3.—*Mean wireworm-population levels obtained by taking samples of 25, 50, and 100 units of 1 square foot, ¼ square foot, and 1/16 square foot in the same field*

1-SQUARE-FOOT UNITS

Field no.	Average wireworms obtained from samples of the specified number of units					
	25		50		100	
	Mean [1]	Relative standard error	Mean [1]	Relative standard error	Mean [1]	Relative standard error
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1					0.22±0.04	18.1
2	0.28±0.09	32.1	0.32±0.08	25.0	.45± .09	20.0
3	.68± .17	25.0	.78± .18	23.1	.69± .11	15.9
4	1.08± .30	27.8	.88± .18	20.5	.74± .11	14.9
5	.92± .20	21.7	.96± .16	16.7	.81± .12	14.8
6	.44± .14	31.8	.82± .17	20.7	.96± .14	14.6
7	1.22± .35	28.7	1.24± .22	17.7	1.25± .18	14.4
8	1.48± .31	20.9	1.44± .25	17.4	1.75± .20	11.4
9	2.56± .50	19.5	3.14± .42	13.4	2.95± .24	8.1
10	3.32± .51	15.4	2.96± .36	12.2	2.98± .34	11.4
11	3.24± .52	16.0	3.10± .38	12.3	3.16± .33	10.4
12	3.00± .72	24.0	3.18± .44	13.8	3.18± .30	9.4
13	4.00± .79	19.8	3.66± .49	13.4	3.92± .39	9.9
14	4.24±1.00	23.6	3.88± .43	11.1	3.94± .38	9.6
15	3.20± .44	13.8	3.40± .37	10.9	3.69± .32	8.7
16	4.12± .48	11.7	4.02± .40	9.9	4.25± .20	4.7
17	4.00± .51	12.8	4.36± .43	9.9	4.71± .37	7.9
18	6.96±1.09	15.7	5.66± .66	11.7	6.01± .47	7.8
19	5.80± .70	12.1	6.76± .80	11.8	6.31± .52	8.2
20	6.96± .90	12.9	7.08± .93	13.1	7.02± .59	8.4
21	8.56±1.30	15.2	7.94± .86	10.1	7.53± .56	7.4
22	9.28±1.19	12.8	8.96± .71	7.9	7.96± .51	6.4
23	8.60±1.23	14.3	7.86± .56	7.1	8.20± .47	5.7
24	11.12±1.63	14.7	7.60± .77	10.1	8.83± .71	8.0
25	14.16±2.12	15.0	9.46± .84	8.9	10.78± .76	7.1
¼-SQUARE-FOOT UNITS						
1	0.20±0.10	50.0	0.12±0.05	41.7	0.12±0.04	33.3
2	.16± .07	44.8	.12± .05	41.7	.12± .03	25.0
3	.40± .10	25.0	.20± .06	30.0	.29± .05	17.2
4	.60± .15	25.0	.52± .10	19.2	.47± .07	14.9
5					.56± .12	21.4
6	1.40± .29	20.7	1.00± .16	16.0	.77± .11	14.3
7	1.08± .28	24.1	1.14± .19	16.7	.90± .11	12.2
8	1.68± .37	22.0	2.08± .26	12.5	1.86± .19	10.2
1/16-SQUARE-FOOT UNITS						
1	0.12±0.06	50.0	0.08±0.04	50.0	0.05±0.02	40.0
2			.08± .04	50.0	.10± .03	30.0
3	.12± .08	66.7	.10± .05	50.0	.11± .03	27.3
4	.16± .07	43.7	.18± .06	33.3	.25± .06	24.0
5	.64± .20	31.3	.46± .10	21.7	.41± .06	14.6
6	.32± .13	25.0	.74± .18	24.3	.50± .10	20.0
7	.60± .16	26.6	.62± .13	21.0	.64± .09	14.1
8	.72± .16	22.2	.66± .11	16.7	.73± .09	12.3
9	.76± .15	19.7	.84± .12	14.3	.88± .10	11.4
10					1.00± .24	24.0
11	1.04± .29	27.9	1.08± .22	20.4	1.13± .14	12.4
12					2.00± .31	15.5
13	1.80± .41	22.8	2.14± .28	13.1	2.19± .24	11.0

[1] See footnote table 2.

To determine the smallest number of units that may be taken to give a mean with a relative standard error considered reasonable for wireworm populations, comparisons were made on the basis of four different population densities for each of the three sampling units. Means and standard deviations were calculated from 100 units of each size from the four different population densities. Considering the standard deviations obtained from these distributions as representative of the true standard deviations of the population, theoretical standard errors of the mean were computed by varying the number of units in the sample from 1 to 100. These errors were converted into relative errors of the mean to facilitate comparison and then plotted in figure 7.

A study of these graphs reveals the more important characteristics shown by the data. Little practical effect upon the sampling error is obtained at any population level by using more than 50 units to determine the average number of wireworms per square foot of soil, but at least 50 units are necessary at low population levels. There is demonstrated also a decreasing necessity for taking 50 units, as the size of the sampling unit is increased from $\frac{1}{16}$ square foot to 1 square foot. At the intermediate and high levels of population a smaller number of units is sufficient.

Except for low population levels, the number of units required for a 20- to 40-percent error range is not over 25 for 1-square-foot and $\frac{1}{4}$-square-foot units, or 50 for $\frac{1}{16}$-square-foot units.

DISCUSSION

There are certain limits to the amount of sampling that is practicable in entomological field surveys. The field man has only a limited amount of time to devote to sampling. The work here reported shows that fifty 1-square-foot units, sifted, are enough to give fairly accurate estimates of the number of wireworms at any population level. These 1-square-foot siftings, taken annually in the same field, permit comparisons of wireworm populations from one season to another. The degree of crop damage done by different numbers of wireworms can be determined. The 1-square-foot unit is large enough to be used where depth records of the wireworms are desired in special studies of vertical migration. For purposes pertaining to the life history, however, where counts of eggs and young larvae are required, or for determining the size of wireworm infestations during summer control practices, the $\frac{1}{16}$-square-foot units, washed, are necessary. Fifty of these units, washed, will give fairly accurate estimates of the population, except for very low population levels, where 100 such units may be necessary.

Each of these sampling units has certain advantages. The technique developed for obtaining wireworm counts by sifting calls for more costly equipment than that for obtaining counts by washing. With each method the labor of two men is required. From 30 to 50 cubic feet of soil per day can be sifted with the power sifter, whereas one hundred $\frac{1}{16}$-square-foot units can be washed and examined in a working day.

SUMMARY

Practical quantitative methods of estimating wireworm populations in soil have been presented. Efforts have been made to find a time- and labor-saving system of sampling that will give fairly accurate

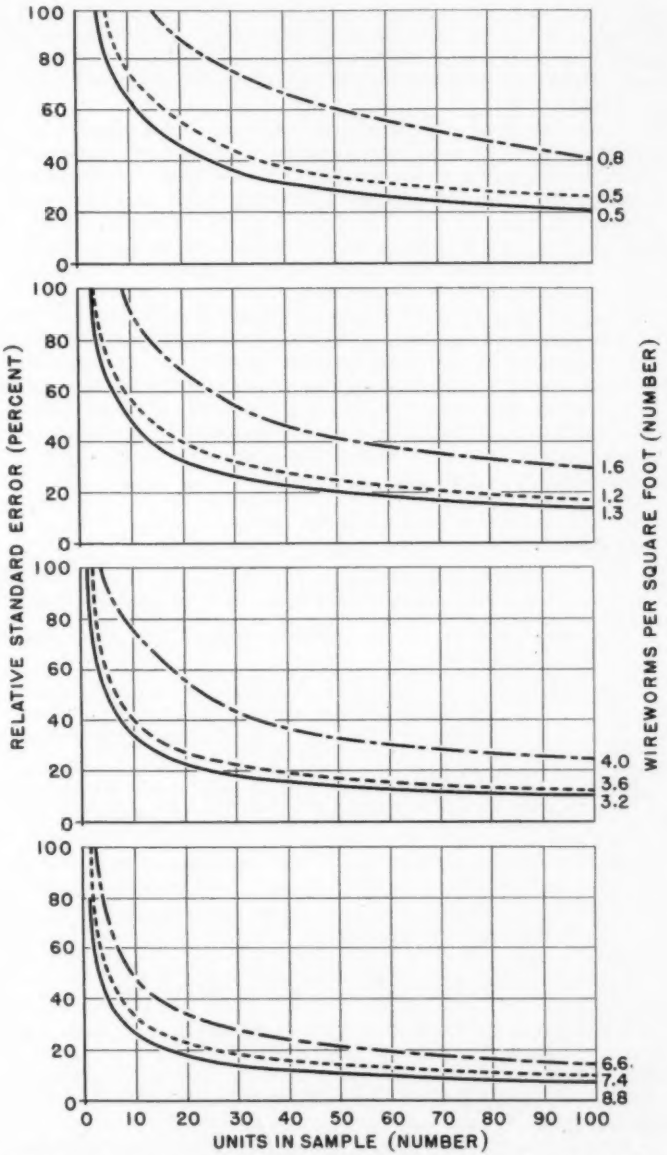

FIGURE 7.—Error in sampling wireworm populations at different densities, expressed in number of wireworms per square foot. Solid lines represent 1-square-foot units, short equal-length dashes ¼-square-foot units, and long and short dashes 1/16-square-foot units.

results. In brief, the methods selected consist of taking small sampling units of soil at random over the field. The field equipment consists of a metal sampling form, a soil-sifting machine or a soil-washing outfit, a tamper, a square-edged shovel, 10-quart pails, and record blanks.

The data obtained with sampling units of three sizes—1, ¼, and 1/16 square foot—have been subjected to a statistical analysis. The wireworm distribution is moderately asymmetrical for the 1-square-foot unit, the class frequency decreasing with markedly greater rapidity on one side of the maximum than on the other. The distributions show close agreement with the Poisson series. It is shown that the 1-square-foot units give more precision in estimating the mean than either of the smaller units.

In determining the smallest number of sampling units that may be taken, comparisons were made on the basis of four different population densities for each of the three units. Little practical reduction of the sampling error is obtained at any population by using more than 50 samples to determine the average number of wireworms per square foot of soil. At low population levels it is necessary to take at least 50 samples. At the intermediate and high levels of population a smaller number of samples is sufficient.

LITERATURE CITED

(1) Edwards, E. E.
1929. A survey of the insect and other invertebrate fauna of permanent pasture and arable land of certain soil types at Aberystwyth. Ann. Appl. Biol. 16: 299–323, illus.

(2) Hawkins, J. W.
1934. Wireworm injury to potatoes. Maine Agr. Expt. Sta. Bull. 377: 354–356.

(3) King, K. M.
1929. The value of quantitative methods in the investigation of field crop insects, with special reference to work with wireworms and cutworms. 4th Internatl. Cong. Ent. (Ithaca) 1928, Trans. v. 2, pp. 248–258, illus.

(4) ——— and Glen, R.
1933. A co-operative investigation of the quantitative relation between summer-fallow methods and the wireworm in Saskatchewan. A progress report. Sci. Agr. 13: 646–652.

(5) Lacroix, D. S.
1935. Tobacco insects in 1934. Conn. Agr. Expt. Sta. Bull. 367: 135–143, illus.

(6) Lane, M. C., and Shirck, F. H.
1928. A soil sifter for subterranean insect investigations. Jour. Econ. Ent. 21: 934–936, illus.

(7) Morris, H. M.
1922. The insect and other invertebrate fauna of arable land at Rothamsted. Ann. Appl. Biol. 9: 282–305, illus.

(8) Pearson, K.
1914. Tables for statisticians and biometricians. 143 pp., illus. Cambridge.

(9) Roebuck, A.
1924. Destruction of wireworms. Jour. Min. Agr. [Gt. Brit.] 30: 1047–1051.

(10) Shirck, F. H.
1930. A soil-washing device for use in wireworm investigations. Jour. Econ. Ent. 23: 991–994, illus.

(11) Wardle, R. A.
1929. The problems of applied entomology. 587 pp., illus. Manchester, England.

THE BIOLOGICAL VALUE OF THE PROTEINS IN HEGARI AND THE SUPPLEMENTAL VALUE OF CERTAIN PROTEIN CONCENTRATES USED IN FARM ANIMAL FEEDING [1]

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INTRODUCTION

Previous work in this laboratory has shown that the sorghum grain hegari, like other grains, is not adequate as the sole source of protein in the diet of rats (*14*).[2] That the protein in grains must be supplemented has long been recognized, and it is common practice to incorporate in farm animal rations made up largely of grains, a protein-rich food such as tankage, cottonseed meal, etc.

Osborne and Mendel (*13*) and McCollum, Simmonds, and Parsons (*1, 2, 3, 4, 5*) have demonstrated the way in which proteins supplement each other in nutrition, and have pointed out that the nutritive value of a protein when fed as the only source of protein in the ration is not a measure of its nutritive value when fed in combination with other proteins. It is a matter of great practical importance, therefore, so to combine natural foods as to secure protein mixtures of high biological value. Thus the question of the best protein concentrate from the standpoint of supplemental value to hegari proteins arises. This paper gives the results of an investigation of the nutritive value of hegari proteins and the extent to which certain protein concentrates exert a supplemental effect.

EXPERIMENTAL PROCEDURE AND RESULTS

GROWTH EXPERIMENTS

In the first series of experiments the nutritive value of hegari as the sole source of protein in a ration, and the nutritive value of hegari supplemented by certain protein concentrates or amino acids, was studied by means of growth experiments. The method followed in part of this investigation was essentially that used by McCollum, Simmonds, and Parsons (*1, 2, 3, 4, 5*). Albino rats were taken at the weaning age (4 weeks) when weighing 45 to 65 g, placed in separate cages, and given a ration containing a single protein food or a mixture of proteins at a level of 9 percent, for McCollum considered this a critical level even when the protein was of excellent quality. The diets were so constituted (table 1) that all the other known dietary essentials were supplied in ample amounts, so that the growth responses would be a measure of the nutritive value of the protein fraction which was the only variable component. Apparently all the rations were palatable to the rats except those containing tankage or meat scrap. The food intake on these rations was low, which may have influenced the utilization of the proteins for growth to some extent.

[1] Received for publication June 30, 1936; issued March 1937.
[2] Reference is made by number (italic) to Literature Cited, p. 145.

TABLE 1.—*Composition of rations used* [1]

Source of protein (approximate values)	Protein by analysis of ration	Hegari	Starch	NaCl	Tankage, 49.2 percent protein	Osborne and Mendel's salt mixture	Cottonseed meal, 41.0 percent protein	Alfalfa-leaf meal, 21.0 percent protein	Meat scrap, 48.6 percent protein	Wheat gluten	Gelatin, 1.4 percent protein	Dried skim milk, 33.6 percent protein	Soybean-oil meal, 42.3 percent protein	Dried egg albumen	Cod-liver oil	Agar
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
9 percent from hegari		91		1		4									2	2
6 percent from hegari, 3 percent from cottonseed meal	9.05	60.7	23.0	1		4	7.3								2	2
9 percent from cottonseed meal	9.35		69.1	1		4	21.9								2	2
6 percent from hegari, 3 percent from soybean-oil meal		60.7	23.2	1		4							7.1		2	2
9 percent from soybean-oil meal			69.7	1		4							21.3		2	2
6 percent from hegari, 3 percent from dried skim milk	8.99	60.7	21.4	1		4						8.9			2	2
9 percent from dried skim milk	9.2		62.2	1		4						26.8			2	4
6 percent from hegari, 3 percent from meat scrap	8.75	60.7	24.1	1		4			6.2						2	2
9 percent from meat scrap	8.9		72.5	1		4			18.5						2	2
6 percent from hegari, 3 percent from tankage		60.7	24.3	1	6.0	4									2	2
9 percent from tankage			73.0	1	18.0	4									2	2
6 percent from hegari, 3 percent from wheat gluten		60.7	27.3	1		4				3.0					2	2
9 percent from wheat gluten			82.0	1		4				9.0					2	2
6 percent from hegari, 3 percent from alfalfa-leaf meal	8.8	60.7	18.0	1		4	14.3								2	
9 percent from alfalfa-leaf meal	9.0		50.1	1		4		42.9							2	
6 percent from hegari, 3 percent from gelatin	8.97	60.7	27.0	1		4						3.3			2	2
9 percent from gelatin			79.2	1		4						9.8			2	4
6 percent from hegari, 3 percent from egg albumen		60.7	27.3	1		4								3	2	2
9 percent from egg albumen			80.0	1		4								9	2	4

[1] Based on analyses of the protein foods made in this laboratory. Vitamin B supplied by extract of rice polishings (tiki tiki), 2 drops daily.

The basal diet and distilled water were available to the animals at all times, and records were made weekly of the food consumed and of the body weights for an 8-week test period.

The protein-rich materials whose value as supplements for hegari proteins was studied included cottonseed meal, soybean-oil meal,[3] dried skim milk, tankage, gelatin, wheat gluten, dried egg albumen, and alfalfa-leaf meal. In each case the protein mixture was fed at a 9-percent level, two-thirds of whch came from hegari and one-third from one of the above-mentioned high-protein foods (table 1).

Table 2 summarizes the results obtained in terms of the average gain in weight and average total food consumed in the 8-week period, and the average gain per gram of protein eaten by the rats on each of the experimental rations. Great differences in value for the support of growth of the various protein foods when fed singly at this critical protein level may be seen. The best gains in weight per gram of food or protein consumed were obtained from dried skim milk and from egg albumen. Cottonseed meal and soybean-oil-meal proteins were somewhat superior to the proteins of wheat gluten, alfalfa-leaf meal, gelatin, and tankage for the support of growth.

Distinct supplementary relations between certain of these proteins and hegari proteins were also observed when they replaced part of the hegari proteins in the ration (i. e., 6 percent of hegari protein and 3 percent of dried skim-milk protein 1). A comparison of experimental gains in weight per gram of protein consumed and the calculated gains in weight which would be expected from a mixture of hegari and the protein concentrate in the proportion fed, revealed a definite supplemental relation in some cases. For example, mixtures of hegari with dried skim milk, or gelatin, or egg albumen, or tankage, or alfalfa-leaf meal provide protein more efficient for the promotion of growth than could be expected if no supplementary relationship existed. It will be seen that when hegari is mixed with dried skim milk, gelatin, egg albumen, alfalfa-leaf meal, or tankage, the actually obtained gains per gram of protein mixture are greater than the weighted mean gain per gram of protein mixture calculated from the gain per gram of each protein in the mixture when fed singly. It would appear therefore that there is a portion of the protein fraction of hegari or of the protein concentrate which is unused when fed singly, but which becomes available when fed in conjunction with each other. The nutritive value of the proteins of gelatin is very poor when fed as the only source of protein in the ration, so much so that rats on this ration cannot even maintain their weight. However when gelatin is combined with hegari, the growth-promoting value of the protein mixture is far superior to either protein when fed singly. The supplementary relations are practically as great between gelatin and hegari proteins as between dried skim milk and hegari proteins. However, it is true, of course, that because of the extremely poor quality of gelatin proteins, the resulting mixture of gelatin and hegari protein at the 9-percent level is markedly inferior in nutritive value to the hegari-milk protein mixture.

[3] The soybean-oil meal used in these tests was a well-cooked, hydraulic-process product.

TABLE 2.—*Comparative efficiency for the support of growth of hegari proteins, when fed at 9 percent protein level for 8 weeks, alone and when supplemented by certain protein concentrates*

Source of protein (approximate values)	Rats	Average initial weight	Average gain in weight	Average total intake		Observed gain in weight per gram of protein ingested	Calculated weighted mean gain in body weight per gram of protein mixture ingested [1]
				Food	Protein		
	Number	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
9 percent from hegari	10	55	24	452	40.68	+0.59	
9 percent from cottonseed meal	4	61	80	550	49.50	+1.62	
6 percent from hegari, 3 percent from cottonseed meal	4	56	69	657	59.13	+1.17	+0.93
9 percent from dried skim milk	4	52	162	677	60.93	+2.65	
6 percent from hegari, 3 percent from dried skim milk	4	52	174	792	71.28	+2.44	+1.28
9 percent from alfalfa-leaf meal	2	58	18	407	36.63	+.49	
6 percent from hegari, 3 percent from alfalfa-leaf meal	4	60	85	655	58.93	+1.44	+.56
9 percent from soybean-oil meal	4	59	40	377	33.9	+1.17	
6 percent from hegari, 3 percent from soybean-oil meal	4	60	70	540	48.6	+1.44	+.78
9 percent from wheat gluten	2	58	50	488	43.92	+1.14	
6 percent from hegari, 3 percent from wheat gluten	2	57	28	350	31.50	+.88	+.77
9 percent from egg albumen	4	63	88	406	36.54	+2.40	
6 percent from hegari, 3 percent from egg albumen	4	56	135	700	63.00	+2.14	+1.19
9 percent from gelatin	4	52	20	248	22.32	+.89	
6 percent from hegari, 3 percent from gelatin	4	56	46	466	41.94	+1.10	+.09
6 percent from hegari, 3 percent from tankage	4	55	7	190	17.1	+.41	
9 percent from tankage	4	58	16	139	12.53	+1.27	+.53

[1] Expected on the assumption that there is no supplemental effect among the proteins in the mixture.

On the other hand, no appreciable supplementary relation between the proteins of hegari and wheat gluten or cottonseed meal could be observed. The value of the incorporation of these proteins in hegari rations would depend only upon an increase in the protein content or quality but not upon any supplementary relationship.

By using the perhaps more quantitative paired-feeding procedure considered desirable by Mitchell, information as to the supplemental effect of certain amino acids has been obtained. For this study the rats were carefully matched as to sex, litter, and initial weight and the food intake of one member of a pair was restricted to the food intake of the other. The rats were weighed weekly and accurate records of the food intake were made daily for a period of 6 weeks. Using the comparative growth response in from 7 to 10 pairs of rats as the criterion, the supplementary relations between hegari and cystine, tryptophane, and lysine were investigated. The amino acid additions in the amount of 0.02 g fed daily were made to the hegari ration used in the previously described experiments. (See table 1.)

The results are presented in table 3, for tryptophane, cystine, and lysine, respectively, and the effect of feeding gelatin as a protein high in lysine is also shown. In the last case, the gelatin provided 3 percent and the hegari 6 percent of the protein in the ration. Inspection of these tabulated results shows that no supplemental effect between cystine and hegari proteins or between tryptophane and hegari pro-

teins could be consistently demonstrated. However, in every case, the addition of lysine accelerated the growth rate of the rats receiving this supplement to their hegari ration. Again, it may be noted in table 3 that when gelatin as a source of lysine was incorporated in the ration, the growth of the gelatin-fed member of the rat pair exceeded the growth rate of its pair mate in every case, thus giving further evidence that lysine is a limiting factor in the hegari-protein make-up.

TABLE 3.—*Effect of the addition of tryptophane, cystine, lysine, and gelatin to hegari proteins, as shown by paired-feeding growth experiments of 6 weeks' duration*

TRYPTOPHANE

Pair no.	Source of protein	Initial weight of rats	Final weight of rats	Total food intake	Gain or loss in weight [1]	Supplemental effect
		Grams	*Grams*	*Grams*	*Grams*	
1	Hegari	43	47	264	4	
	Hegari +0.02 g tryptophane daily	42	43	263	1	−
2	Hegari	60	69	295	9	
	Hegari +0.02 g tryptophane daily	48	56	298	8	−
3	Hegari	48	49	254	1	
	Hegari +0.02 g tryptophane daily	48	52	253	4	+
4	Hegari	53	58	224	5	
	Hegari +0.02 g tryptophane daily	55	61	224	6	+
5	Hegari	60	69	267	9	
	Hegari +0.02 g tryptophane daily	60	69	265	9	0
6	Hegari	63	74	271	11	
	Hegari +0.02 g tryptophane daily	61	75	271	14	+
7	Hegari	55	66	258	11	
	Hegari +0.02 g tryptophane daily	68	76	256	8	−
8	Hegari	79	71	270	12	
	Hegari +0.02 g tryptophane daily	58	67	270	9	−
Average	Hegari				7.7	
	Hegari +0.02 g tryptophane daily				7.4	−

CYSTINE

1	Hegari	63	73	268	10	
	Hegari +0.02 g cystine daily	61	70	268	9	−
2	Hegari	57	68	265	11	
	Hegari +0.02 g cystine daily	56	71	264	15	+
3	Hegari	70	81	254	11	
	Hegari +0.02 g cystine daily	69	77	258	8	−
4	Hegari	65	74	267	9	
	Hegari +0.02 g cystine daily	65	75	264	10	+
5	Hegari	59	67	260	8	
	Hegari +0.02 g cystine daily	58	68	253	10	+
6	Hegari	59	76	282	17	
	Hegari +0.02 g cystine daily	60	75	284	15	−
7	Hegari	60	77	277	17	
	Hegari +0.02 g cystine daily	59	71	277	12	−
Average	Hegari				11.9	
	Hegari +0.02 g cystine daily				11.3	

LYSINE

1	Hegari	60	45	187	−15	
	Hegari +0.02 g lysine daily	60	58	186	−2	+
2	Hegari	56	62	229	6	
	Hegari +0.02 g lysine daily	57	71	232	14	+
3	Hegari	55	59	257	4	
	Hegari +0.02 g lysine daily	55	65	262	10	+
4	Hegari	60	62	241	2	
	Hegari +0.02 g lysine daily	61	73	240	12	+
Average	Hegari				−.8	
	Hegari +0.02 g lysine daily				8.5	+

[1] Minus sign (−) indicates a loss.

TABLE 3.—*Effect of the addition of tryptophane, cystine, lysine, and gelatin to hegari proteins, as shown by paired-feeding growth experiments of 6 weeks' duration*—Con.

GELATIN

Pair no.	Source of protein	Initial weight of rats	Final weight of rats	Total food intake	Gain or loss in weight	Supplemental effect
		Grams	*Grams*	*Grams*	*Grams*	
1	Hegari	55	69	283.5	14	
	Hegari +0.02 g gelatin daily	56	78	281.0	22	+
2	Hegari	55	74	310.0	19	
	Hegari +0.02 g gelatin daily	53	78	309.0	25	+
3	Hegari	60	80	313.0	20	
	Hegari +0.02 g gelatin daily	61	84	315.0	23	+
4	Hegari	48	64	268.0	16	
	Hegari +0.02 g gelatin daily	50	75	266.5	25	+
5	Hegari	55	65	772.0	10	
	Hegari +0.02 g gelatin daily	53	70	274.0	17	+
6	Hegari	58	68	284.0	10	
	Hegari +0.02 g gelatin daily	58	71	281.0	13	+
7	Hegari	60	65	254.0	5	
	Hegari +0.02 g gelatin daily	59	72	252.5	13	+
8	Hegari	61	73	276.0	12	
	Hegari +0.02 g gelatin daily	60	80	278.0	20	+
9	Hegari	57	71	307.0	14	
	Hegari +0.02 g gelatin daily	56	76	301.0	20	+
10	Hegari	59	75	311.0	16	
	Hegari +0.02 g gelatin daily	59	78	307.0	19	+
Average	Hegari				13.6	
	Hegari +0.02 g gelatin daily				19.7	+

NITROGEN-BALANCE EXPERIMENTS

In the second series of experiments planned to determine the nutritive value of the proteins of hegari and its supplementary relations with certain protein-rich foods, the more direct method of investigation by means of nitrogen-metabolism studies was followed.

This method, based upon that used by Karl Thomas, has been worked out in great detail by Mitchell (*6*, *8*) for use with rats. It consists essentially in measuring the percentage of the absorbed food nitrogen that is retained in the body, the result being referred to as the biological value of the protein.

Male albino rats only were used in the experiments. They were housed in galvanized-iron cages with raised screen bottoms placed upon porcelain plates. Distilled water was provided in a glass bottle attached to the outside of the cage. The analyzed food rations were placed in nonscatter glass cups and a quantitative record of the food intake was made. The urine was absorbed on large circles of filter paper which had previously been soaked in a 1 to 1 solution of hydrochloric acid and dried. The filter paper with absorbed urine was changed daily and the feces collected quantitatively and preserved with concentrated sulphuric acid. At the end of the 5-day test period, which had been preceded by a 3-day fore period for dietary adjustment, the filter papers were washed free from urine with hot, dilute hydrochloric acid, made up to volume, and an aliquot was taken for nitrogen analysis by the Kjeldahl method. All the feces obtained in the test period were digested with concentrated sulphuric acid, mercury, and potassium sulphate, diluted and made up to volume, and an aliquot was analyzed for nitrogen.

For each rat, the endogenous nitrogen in urine and in feces (per 100 g of body weight and per gram of food consumed respectively) was determined on the low-nitrogen whole-dried egg diet recommended by Mitchell and Carman (*9*), except that the lard was omitted and the

percentage of carbohydrate correspondingly increased. As hegari rations supplying approximately 9 percent of hegari proteins must of necessity be largely hegari, this adjustment was made in order that the energy value of all the experimental rations used for nitrogen-balance studies should be approximately the same.

The experimental rations were again those shown in table 1. They were so planned as to be approximately the same not only in energy value but also in roughage content.

The results of these nitrogen-balance studies expressed as biological value of the proteins when fed singly and also in mixture with hegari proteins appear in tables 4 and 5.

It may be seen that hegari proteins have the relatively low biological value of 53.2 percent. About half of the absorbed protein in hegari, therefore, is not utilizable for growth, because the assortment of amino acids essential for synthesis into the complex body tissue is not complete. Thus hegari protein is capable of being supplemented to a large extent by those proteins that supply the amino acids which are deficient in the hegari protein make-up.

The biological value of dried skim milk, alfalfa-leaf meal, cottonseed meal, and soybean-oil meal as determined in this laboratory agree closely with the results of similar measurements made by Mitchell and others (*7*, *10*, *11*, *13*, *15*). Determination of the biological value of these protein foods when fed singly and in combination to the same group of animals leaves no doubt as to the reliability of the observed supplemental values.

The supplementary relations between hegari protein and the proteins of dried skim milk, cottonseed meal, meat scrap, alfalfa-leaf meal, and soybean-oil meal are shown in table 5. The differences between the true or observed biological value of the mixture and the calculated biological value, which is the weighted mean of the biological values of the proteins in the mixture when fed separately, gives the supplemental value of the proteins concerned.

TABLE 4.—*Biological value of proteins from various sources when fed singly and in mixture with hegari proteins*

HEGARI PROTEINS FED AT A 9 PERCENT LEVEL

Rat no.	Initial weight	Final weight	Daily food intake	Nitrogen intake	Fecal nitrogen	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Food nitrogen in urine	Absorbed nitrogen retained in body	Biological value of protein
	G	*G*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Pct.*
12406	162	165	14.0	180.8	26.94	7.90	173.0	105.48	79.9	93.1	53.8
12408	163	164	15.2	196.3	38.46	11.26	185.0	112.95	80.3	104.7	56.6
12409	156	159	18.0	232.3	44.99	32.07	200.0	132.66	95.8	104.2	52.1
12410	149	149	13.6	175.6	27.13	6.0	169.6	100.46	68.7	100.9	59.5
11893	197	201	14.8	190.9	48.4	17.8	173.1	112.6	94.1	79.0	45.6
12246	235	238	13.4	172.9	34.4	11.9	161.0	102.5	62.8	98.2	60.9
12415	254	261	17.0	219.3	45.4	19.4	199.9	128.2	85.3	114.6	57.3
12418	212	217	15.6	201.2	40.9	15.0	186.2	129.9	92.4	93.8	50.4
8659	151	159	10.2	142.6	36.5	30.6	112.0	91.3	55.1	56.9	50.8
8676	158	159	7.8	109.0	25.2	17.6	91.5	75.5	40.0	51.5	56.3
8678	159	160	8.0	111.8	26.8	19.4	92.4	74.3	38.0	54.5	59.0
8684	147	152	8.8	123.0	28.1	21.8	101.2	79.5	46.9	54.3	53.7
8685	150	151	6.8	95.1	23.9	20.5	74.6	63.1	30.1	44.5	59.7
12111	282	285	11.0	153.8	37.6	27.4	126.4	101.5	62.7	63.7	51.4
12112	276	278	19.2	128.6	35.2	23.8	104.8	93.2	55.7	49.1	46.9
12113	261	259	8.8	123.0	31.8	20.5	102.5	93.0	55.5	47.0	45.9
12115	285	282	9.8	139.1	37.6	26.3	112.8	96.6	58.6	54.2	48.0
12114	292	292	10.8	151.0	45.0	32.7	112.8	98.8	60.6	57.8	48.9
Average											53.2

TABLE 4.—*Biological value of proteins from various sources when fed singly and in mixture with hegari proteins*—Continued

COTTONSEED PROTEINS FED AT A 9-PERCENT LEVEL

Rat no.	Initial weight	Final weight	Daily food intake	Nitrogen intake	Fecal nitrogen	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Food nitrogen in urine	Absorbed nitrogen retained in body	Biological value of protein
	G	*G*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Pct.*
12406	173.0	175.0	13.2	197.46	54.2	35.7	161.8	80.6	53.3	108.5	67.1
12407	186.0	187.0	12.5	187.0	47.0	23.3	163.7	84.0	45.5	118.2	72.2
12408	175.5	174.5	13.0	194.4	61.0	37.6	156.8	97.4	62.3	94.5	60.3
12409	170.0	171.0	13.4	200.4	38.9	29.5	170.9	90.7	50.8	120.1	70.3
12410	150.5	151.6	10.4	155.5	42.7	26.1	129.4	73.9	41.7	87.7	67.8
10059	268.0	270.0	10.0	149.9	42.4	32.3	117.6	95.2	56.4	61.2	52.0
10076	258.0	264.0	10.0	149.9	39.3	26.8	123.1	85.1	47.6	75.5	61.3
10078	237.0	245.0	8.0	119.9	32.8	21.5	98.4	71.3	33.8	64.6	65.7
10084	265.0	276.0	9 8	146.9	38.4	26.2	120.7	85.6	47.4	73.4	60.8
10085	210.0	248.0	11.0	164.9	43.4	31.7	133.2	89.5	58.9	74.3	55.8
Average											63.3

MIXTURE OF HEGARI AND COTTONSEED-MEAL PROTEINS FED AT A 9-PERCENT LEVEL, ⅔ HEGARI PROTEIN AND ⅓ COTTONSEED-MEAL PROTEIN

8659	281.0	284.0	12.0	167.2	40.8	30.6	136.6	105.6	66.9	69.7	51.0
8676	266.0	270.0	9.4	131.1	35.2	22.8	108.3	84.4	46.9	61.4	56.7
8678	250.0	252.0	9.8	136.9	35.2	23.9	113.0	93.3	55.8	57.2	50.6
8684	284.0	284.0	9.8	122.9	24.4	22.1	100.8	93.3	55.1	45.7	45.3
8685	253.0	254.0	9.6	134.0	32.7	21.0	113.0	77.2	46.6	66.4	58.8
12406	192.5	197.0	15.2	196.1	29.4	8.7	187.4	80.6	51.4	136.0	72.6
12407	205.0	201 0	15.5	199.9	62.2	33.4	166.5	106.9	75.1	91.4	54.9
12408	180.5	189.0	15.6	201.2	65.0	37.1	164.1	104.2	76.1	88.0	53.6
12409	182.0	194.5	17.0	219.3	54.6	42.9	176.4	96.9	66.0	110.4	62.
12410	153.0	159.0	14.1	181.9	63.0	40.4	141.5	95.2	73.6	67.9	48.0
12411	141.5	140.0	7.2	92.9	20.4	8.9	84.0	42.6	32.4	51.6	61.4
Average											56.0

DRIED SKIM-MILK PROTEINS FED AT A 9-PERCENT LEVEL

12406	206.0	208.0	15.0	210.0	35.3	22.3	187.7	81.1	30.4	157.3	83.8
12407	216.0	217.0	15.9	222.6	49.0	11.5	211.1	76.7	37.4	173.7	82.3
12408	196.0	205.0	14.4	201.6	53.9	26.4	175.2	64.4	31.1	144.1	82.2
12409	196.0	204.0	15.4	215.6	46.3	35.7	179.9	75.0	29.0	150.9	83.9
12410	167.0	188.0	13.0	182.0	45.9	26.8	155.2	57.1	4.5	150.7	97.1
12411	152.0	162.0	12.8	179.2	28.1	12.4	166.8	65.0	27.6	139.2	83.5
12246	237.0	238.0	11.8	165.2	39.6	19.3	145.9	73.4	33.4	112.5	77.1
12415	254.0	259.0	14.0	196.0	39.9	18.5	177.5	92.4	47.5	130.0	73.2
Average											82.9

MIXTURE OF HEGARI AND DRIED SKIM-MILK PROTEINS FED AT A 9-PERCENT LEVEL, ⅔ HEGARI PROTEIN AND ⅓ DRIED SKIM-MILK PROTEIN

12407	238.0	247.0	19.0	273.6	53.3	8.7	264.9	115.9	72.6	192.3	72.6
12408	215.0	226.0	19.3	277.9	42.0	5.1	272.8	105.8	69.3	203.5	74.6
12409	213.0	224.0	18.6	257.8	48.3	35.5	232.3	113.6	63.6	168.7	72.6
12410	181.0	190.0	16.5	237.6	59.2	35.3	202.3	90.7	33.7	168.6	83.3
12411	169.0	184.0	15.2	218.9	37.4	18.7	200.2	87.9	46.3	153.9	76.9
11983	197.0	202.0	13.8	198.7	44.0	15.4	183.3	84.6	27.5	155.8	85.0
12246	232.0	243.0	14.6	210.2	40.5	15.4	194.8	76.5	37.3	157.5	80.9
12415	240.0	250.0	16.0	230.4	54.9	30.4	200.0	96.3	55.7	144.3	72.2
12418	219.0	217.0	11.8	169.9	28.0	8.4	161.5	75.6	35.8	124.7	77.2
8659	171.0	182.0	9.8	142.8	31.1	25.2	117.6	61.6	25.4	92.2	78.4
8676	177.0	186.0	9.1	132.6	24.6	17.0	115.5	57.9	22.3	93.3	80.8
8678	177.0	180.0	8.5	123.8	26.8	19.3	104.5	61.4	25.1	79.5	76.1
8684	178.0	179.0	9.6	139.9	35.2	28.8	111.1	65.7	33.1	78.0	70.2
8685	160.0	170.0	10.0	145.7	29.1	24.4	121.3	65.2	22.2	99.1	81.7
Average											77.3

TABLE 4.—*Biological value of proteins from various sources when fed singly and in mixture with hegari proteins*—Continued

ALFALFA-LEAF-MEAL PROTEINS FED AT A 9-PERCENT LEVEL

Rat no.	Initial weight	Final weight	Daily food intake	Nitrogen intake	Fecal nitrogen	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Food nitrogen in urine	Absorbed nitrogen retained in body	Biological value of protein
	G	*G*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Pct.*
12408	209.0	209.0	11.0	158.4	20.2	------	158.4	85.7	50.2	108.2	68.3
12410	180.0	181.0	9.2	132.5	56.4	43.1	89.4	91.3	34.6	54.8	61.3
12411	170.0	164.0	6.8	97.9	38.6	30.2	67.7	68.3	26.5	41.2	60.9
12406	247.0	243.0	15.4	221.8	93.5	70.4	151.4	103.0	68.2	83.2	55.0
12407	266.0	264.0	19.8	285.1	106.7	70.7	214.4	124.9	77.3	137.1	63.9
12409	259.0	251.0	15.8	227.5	59.9	36.7	190.8	122.1	70.3	120.5	63.2
12410	212.0	205.0	14.0	201.6	98.3	79.0	122.6	107.5	61.3	61.3	50.0
12411	210.0	208.0	13.8	198.7	51.5	31.6	167.1	95.8	53.8	113.3	67.8
11983	200.0	200.0	12.8	179.2	40.8	14.3	164.9	99.1	66.1	98.8	59.9
8676	181.0	192.0	11.9	184.4	22.7	15.2	169.3	99.9	64.4	104.9	62.0
Average	------	------	------	------	------	------	------	------	------	------	61.2

MIXTURE OF HEGARI AND ALFALFA-LEAF-MEAL PROTEINS FED AT A 9-PERCENT LEVEL, ⅔ HEGARI PROTEIN AND ⅓ ALFALFA-LEAF-MEAL PROTEIN

12406	225.0	227.0	19.6	245.0	53.5	36.8	208.2	111.4	77.9	130.3	62.6
12407	225.0	242.0	22.2	277.5	59.2	6.9	270.6	132.2	91.3	179.3	66.3
12408	213.0	223.0	19.6	245.0	64.0	26.6	218.4	111.4	75.2	143.2	65.6
12409	212.0	232.0	22.2	277.5	57.9	42.6	234.9	130.5	80.7	154.2	65.6
12410	180.0	184.0	16.6	207.5	57.3	33.2	174.3	100.2	43.5	130.8	75.0
12411	174.0	187.0	17.4	217.5	41.3	19.9	197.6	101.4	58.6	139.0	70.3
12408	248.0	253.0	18.4	231.8	70.6	35.5	196.3	101.9	59.7	136.6	69.6
12411	205.0	211.0	15.0	189.0	56.0	37.5	151.5	91.8	41.4	110.1	72.7
12421	200.0	204.0	11.2	156.8	29.7	13.0	143.8	82.9	49.7	94.1	65.4
8684	170.0	166.0	9.5	147.2	21.6	15.2	132.0	80.1	47.6	84.5	64.0
Average	------	------	------	------	------	------	------	------	------	------	67.7

MEAT-SCRAP PROTEINS FED AT A 9-PERCENT LEVEL

12411	192.0	191.0	9.8	185.2	39.8	25.7	159.5	115.9	86.1	73.4	46.0
12407	249.0	241.0	15.8	298.6	90.0	52.7	245.9	174.2	128.9	117.0	47.5
12408	233.0	230.0	16.6	313.7	91.4	59.7	254.0	182.0	142.4	111.6	43.9
12409	235.0	235.0	16.8	317.5	90.2	78.6	238.9	182.6	127.4	111.5	46.7
14206	236.0	233.0	13.8	260.8	59.1	38.4	222.4	152.3	117.1	105.3	47.3
11983	203.0	198.0	12.0	226.8	66.8	41.7	185.1	143.5	109.0	76.1	41.1
12407	269.0	264.0	12.2	230.6	58.8	36.6	194.0	151.8	103.7	90.3	46.5
12246	233.0	231.0	12.0	226.8	41.1	20.5	206.3	152.9	113.5	92.8	45.0
12415	255.0	235.0	7.6	143.6	26.2	14.6	129.0	118.2	75.1	53.9	41.8
12418	209.0	203.0	11.8	223.3	43.6	24.6	198.7	149.0	112.0	86.7	43.6
12421	202.0	201.0	11.4	215.5	37.3	20.3	195.2	133.8	100.3	95.0	48.7
8659	168.0	157.0	14.0	264.6	70.8	44.8	219.8	159.0	112.7	107.1	48.7
8676	161.0	160.0	8.2	121.6	33.8	26.2	95.4	80.4	44.8	50.6	53.0
8678	169.0	161.0	7.2	106.8	36.6	29.1	77.7	72.6	36.2	41.4	53.3
8684	156.0	152.0	8.0	118.6	34.8	28.5	90.1	86.1	43.5	46.6	51.7
8085	154.0	148.0	5.0	74.2	22.7	18.0	56.2	59.6	26.6	29.6	52.7
12410	194.0	203.0	17.8	336.4	82.0	56.2	280.2	168.0	106.9	173.3	61.8
Average	------	------	------	------	------	------	------	------	------	------	48.2

TABLE 4.—*Biological value of proteins from various sources when fed singly and in mixture with hegari proteins*—Continued

MIXTURE OF HEGARI AND MEAT-SCRAP PROTEINS FED AT A 9-PERCENT LEVEL, ⅔ HEGARI PROTEIN AND ⅓ MEAT-SCRAP PROTEIN

Rat no.	Initial weight	Final weight	Daily food intake	Nitrogen intake	Fecal nitrogen	Food nitrogen in feces	Absorbed nitrogen	Urinary nitrogen	Food nitrogen in urine	Absorbed nitrogen retained in body	Biological value of protein
	G	*G*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Pct.*
12406	238.0	247.0	18.6	186.0	43.3	27.5	158.5	111.4	75.9	82.6	52.1
12407	250.0	256.0	20.2	202.0	36.3	0	202.0	107.5	62.0	140.0	69.3
612408	230.0	238.0	20.0	200.0	46.5	8.3	191.7	103.0	63.9	127.8	66.7
11983	200.0	200.0	15.8	158.0	47.0	14.3	143.7	96.9	62.9	80.8	56.2
12246	235.0	240.0	16.6	166.0	50.7	22.1	143.9	100.8	61.1	82.8	57.5
12418	213.0	216.0	17.4	174.0	56.6	25.8	148.2	108.6	70.9	77.3	52.2
12421	204.0	200.0	16.2	162.0	52.9	26.0	136.0	82.3	48.4	87.6	64.4
8684	158.0	161.0	10.0	139.5	28.3	22.0	117.6	75.3	42.7	74.9	63.7
8659	165.0	164.0	8.2	114.4	32.1	26.2	88.2	70.8	34.6	53.6	60.8
8676	167.0	167.0	7.6	106.0	32.9	24.3	81.7	64.2	28.6	53.1	65.0
Average											60.8

SOYBEAN-OIL-MEAL PROTEINS FED AT A 9-PERCENT LEVEL

15008	152.0	157.0	7.2	109.5	21.7	5.9	103.7	80.9	37.2	66.5	64.1
16088	116.0	122.0	10.9	166.8	33.2	21.4	145.4	60.4	49.6	95.7	65.8
16089	118.0	127.0	11.0	168.3	32.6	21.5	146.8	60.6	47.9	98.8	65.2
15007	192.0	200.0	12.7	194.3	15.2	.5	193.8	84.3	57.8	136.0	70.2
16191	113.0	117.0	10.2	154.5	26.5	15.9	138.7	64.6	48.2	90.5	65.2
16192	86.0	94.0	6.0	91.8	9.58	.0	91.8	64.2	41.2	50.6	55.2
Average											64.3

MIXTURE OF HEGARI AND SOYBEAN-OIL-MEAL PROTEINS FED AT A 9-PERCENT LEVEL, ⅔ HEGARI PROTEIN AND ⅓ SOYBEAN-OIL-MEAL PROTEIN

15008	166.0	170.0	18.0	278.0	50.6	10.7	267.3	128.8	81.2	186.1	69.6
16088	111.0	111.0	10.3	175.0	32.7	21.6	153.4	163.9	66.3	87.1	56.8
16089	105.0	109.0	11.0	170.0	30.7	19.6	150.4	76.8	65.6	84.8	56.4
16044	82.0	86.0	6.3	97.0	17.3	10.2	86.85	55.95	22.9	63.9	73.6
15007	192.0	192.0	12.9	193.3	33.1	12.2	181.1	92.9	67.2	113.9	62.9
16091	125.0	134.0	13.4	207.0	29.6	2.9	204.1	66.6	49.2	154.9	75.8
16191	98.0	103.0	12.4	191.6	28.3	17.8	173.8	59.7	45.3	128.5	73.9
16192	82.0	94.0	10.6	163.8	22.6	0.7	163.1	59.7	41.6	121.4	74.5
Average											67.9

TABLE 5.—*Supplementary relations between proteins of hegari and those of cottonseed meal, soybean-oil meal, dried skim milk, meat scrap, or alfalfa-leaf meal as shown by nitrogen-balance experiments*

Source of protein (approximate values)	Tests	Experimental average biological value [1]	Calculated biological value of mixture	Supplemental value
	Number	*Percent*	*Percent*	*Percent*
9 percent from hegari	18	53.2±0.8		
9 percent from cottonseed meal	10	63.3±1.4		
6 percent from hegari, 3 percent from cottonseed meal	11	56.0±1.5	56.6	0.6
9 percent from dried skim milk	8	82.9±1.4		
6 percent from hegari, 3 percent from dried skim milk	14	77.3± .8	63.3	14.0
9 percent from alfalfa-leaf meal	10	61.2±1.2		
6 percent from hegari, 3 percent from alfalfa-leaf meal	10	67.7± .9	55.9	11.8
9 percent from soybean-oil meal	6	64.3±1.35		
6 percent from hegari, 3 percent from soybean-oil meal	8	67.9±1.9	57.0	10.9
9 percent from meat scrap	17	48.2± .8		
6 percent from hegari, 3 percent from meat scrap	10	60.8±1.3	51.6	9.2

[1] ± Probable error.

Thus it is evident that dried skim milk, alfalfa-leaf meal, soybean-oil meal, and meat scrap exhibit supplementary value in the order given, whereas cottonseed meal has no appreciable supplemental effect. The use of cottonseed meal as a protein concentrate in rations made up largely of hegari serves therefore only to increase the total protein in the diet.

Dried skim milk, alfalfa-leaf meal, and meat scrap, on the other hand, may be used not only to increase the total protein content of the ration but also to make available an otherwise nonutilizable portion of the proteins in the mixture, thus serving in two ways to increase the protein in the ration. It would appear that the most efficient proteins from the standpoint of supplemental value for hegari proteins are those rich in lysine.

CONCLUSIONS

The following conclusions have been drawn from growth- and nitrogen-balance experiments with rats fed rations in which hegari served as the only source of protein in an otherwise adequate ration, or was partly substituted with certain protein concentrates:

(1) Hegari proteins are inadequate for the support of growth, as shown by a relatively low rate of gain per gram of protein consumed by the rats used in the growth studies, and a low biological value of the protein as determined by nitrogen-balance experiments.

(2) The protein-rich foods which have shown definite supplemental relations with hegari proteins include dried skim milk, alfalfa-leaf meal, soybean-oil meal, meat scrap, gelatin, and tankage.

(3) The protein concentrates studied which were found to be without supplemental relations were cottonseed meal and wheat gluten.

(4) Paired feeding experiments showed that the amino acid, lysine, has a marked ability to supplement hegari proteins, whereas tryptophane and cystine have not. It would appear that lysine deficiency is at least partly responsible for the low biological value of the proteins of hegari and that the proteins which are rich in this amino acid have a strong supplemental value for hegari.

LITERATURE CITED

(1) McCollum, E. V., Simmonds, N., and Parsons, H. T.
1921. SUPPLEMENTARY PROTEIN VALUES IN FOODS. I. THE NUTRITIVE PROPERTIES OF ANIMAL TISSUES. Jour. Biol. Chem. 47: 111–137, illus.

(2) ——— Simmonds, N., and Parsons, H. T.
1921. SUPPLEMENTARY PROTEIN VALUES IN FOODS. II. SUPPLEMENTARY DIETARY RELATIONS BETWEEN ANIMAL TISSUES AND CEREALS AND LEGUME SEEDS. Jour. Biol. Chem. 47: 139–173, illus.

(3) ——— Simmonds, N., and Parsons, H. T.
1921. SUPPLEMENTARY PROTEIN VALUES IN FOODS. III. THE SUPPLEMENTARY DIETARY RELATIONS BETWEEN THE PROTEINS OF THE CEREAL GRAINS AND THE POTATO. Jour. Biol. Chem. 47: 175–206, illus.

(4) ——— Simmonds, N., and Parsons, H. T.
1921. SUPPLEMENTARY PROTEIN VALUES IN FOODS. IV. THE SUPPLEMENTARY RELATIONS OF CEREAL GRAIN WITH CEREAL GRAIN; LEGUME SEED WITH LEGUME SEED; AND CEREAL GRAIN WITH LEGUME SEED; WITH RESPECT TO IMPROVEMENT IN THE QUALITY OF THEIR PROTEINS. Jour. Biol. Chem. 47: 207–234, illus.

(5) McCollum, E. V., Simmonds, N., and Parsons, H. T.
1921. SUPPLEMENTARY PROTEIN VALUES IN FOODS. V. SUPPLEMENTARY RELATIONS OF THE PROTEINS OF MILK FOR THOSE OF CEREALS AND OF MILK FOR THOSE OF LEGUME SEEDS. Jour. Biol. Chem. 47: 235–247, illus.
(6) Mitchell, H. H.
1924. A METHOD OF DETERMINING THE BIOLOGICAL VALUE OF PROTEIN. Jour. Biol. Chem. 58: 873–903.
(7) ———
1924. THE BIOLOGICAL VALUE OF PROTEINS AT DIFFERENT LEVELS OF INTAKE. Jour. Biol. Chem. 58: 905–922.
(8) ———
1924. THE SUPPLEMENTARY RELATIONS AMONG PROTEINS. Jour. Biol. Chem. 58: 923–929.
(9) ——— and Carman, G. G.
1926. THE BIOLOGICAL VALUE OF THE NITROGEN OF MIXTURES OF PATENT WHITE FLOUR AND ANIMAL FOODS. Jour. Biol. Chem. 68: 183–215.
(10) ——— and Villegas, V.
1923. THE NUTRITIVE VALUE OF THE PROTEINS OF COCONUT NUT MEAL, SOY BEANS, RICE BRAN AND CORN. Jour. Dairy Sci. 6: 222–236.
(11) Nevens, W. B.
1921. THE PROTEINS OF COTTONSEED MEAL. II. NUTRITIVE VALUE. Jour. Dairy Sci. 4: 552–588.
(12) Osborne, T. B., and Mendel, L. B.
1914. AMINO-ACIDS IN NUTRITION AND GROWTH. Jour. Biol. Chem. 17: 325–349, illus.
(13) ——— and Mendel, L. B.
1917. THE RELATIVE VALUE OF CERTAIN PROTEINS AND PROTEIN CONCENTRATES AS SUPPLEMENTS TO CORN GLUTEN. Jour. Biol. Chem. 29: 69–92, illus.
(14) Smith, M. C.
1930. THE COMPARATIVE NUTRITIVE VALUE OF YELLOW CORN AND THE GRAIN SORGHUM HEGARI AND YELLOW MILO. Jour. Agr. Research 40: 1129–1145, illus.
(15) Turk, K. L., Morrison, F. B., and Maynard, L. A.
1935. THE NUTRITIVE VALUE OF THE PROTEINS OF CORN-GLUTEN MEAL, LINSEED MEAL, AND SOYBEAN-OIL MEAL. Jour. Agr. Research 51: 401–412.

PRODUCTS OF FERMENTATION OF THE S AND R FORMS OF YEASTS[1]

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INTRODUCTION

In the course of a study with dissociated forms of yeasts it was noticed that during the process of fermentation certain forms produced a pronounced odor of esters in the fermenting solutions. The question naturally arose as to whether the products of fermentation of the R forms differed materially either qualitatively or quantitatively from the products of fermentation of the corresponding S forms. An experiment was therefore set up to determine the amounts of certain products present at different times during the process. Yeasts which had been dissociated previously [2] were used in this experiment.

CULTURES USED

The cultures used are described below:

S form of Saccharomyces aceris-sacchari Fabian and Hall.—This yeast produces a glistening, moist growth on agar slants and convex colonies on agar plates. The growth is mainly at the bottom in liquid media, although a thin film forms on the surface after a few days' growth. The cells average 3.5 to 7 microns in diameter, are spherical, and are uniform in appearance.

R form of Saccharomyces aceris-sacchari.—This yeast produces a rugose, white, dry growth on agar slants and flat colonies on agar plates. It produces abundant growth on the surface of liquid media and a moderate growth at the bottom. The cells average 10 to 16 microns in length and vary in shape, some being round and others elongate. The individual cells do not present a uniform appearance as do the S cells, for the R cells contain one or more droplets and a large central vacuole.

S form of Pichia alcoholophila Klöcker.—This yeast produces a glistening, moist growth on agar slants and convex colonies on agar plates. It is a bottom yeast. The cells average 6 microns in diameter and are spherical or slightly elongate. The S cells occasionally show small oil droplets.

R form of Pichia alcoholophila.—This yeast produces a dull, wrinkled growth on agar slants and a raised, rugose colony on agar plates. It produces an abundant growth both on the surface and at the bottom of liquid media. The cells average 10 to 20 microns in length. They contain large vacuoles and oil droplets.

[1] Received for publication Apr. 27, 1936; issued March 1937. Some of the data in this paper were taken from a thesis by the junior author presented in partial fulfillment of the requirements for the degree of master of science, Michigan State College. Journal article no. 244 (N. S.).

[2] WICKERHAM, L. J., and FABIAN, F. W. DISSOCIATION OF SACCHAROMYCES, FABIAN AND HALL, and PICHIA ALCOHOLOPHILA KLÖCKER. Jour. Infect. Diseases 58: 165-171. 1936.

S form of Saccharomyces cerevisiae Hansen, Saaz strain.—This yeast, which for the sake of brevity is referred to in this paper as *Saccharomyces cerevisiae* Saaz, is an industrial strain that produces a bottom alcoholic fermentation. The colonies on agar plates are convex, and agar slant cultures have a moist, glistening appearance The cells are spherical and average 6 microns in diameter.

R form of Saccharomyces cerevisiae Saaz.—This yeast produces a wrinkled, white growth on agar slants and a raised, rugose colony. The growth is mainly at the surface of liquid media, although there is moderate growth at the bottom. The cells are 14 to 20 microns in length and vary considerably in shape. Like other R forms, they contain oil droplets and large vacuoles.

SR form of Willia anomala Saito.—The S form of this yeast was first obtained by Fabian and McCullough [3] from the naturally occurring R form in 1933. It was put away in a stock collection, and when it was cultured again for use in this experiment it showed partial reversion to the R form. It produces a colony which is less wrinkled than the true R, is gray instead of white, and appears slightly moist. It produces a thin scum on liquid media. Because it has characteristics of both types it is designated the SR form.

R form of Willia anomala.—This yeast produces a white, rugose growth on agar slants. The colonies are rugose and raised. The cells range in length from 14 to 20 microns. The growth in liquid media is abundant at the surface and moderate at the bottom. The cells show great variation in shape. Only a small percentage of them contain oil droplets, but all contain large vacuoles.

MEDIA USED

Three media were used: Cider, malt-extract broth, and a synthetic medium. The cider had an initial Balling reading of 12°, which was increased to 22° when sucrose was added in order to supply sufficient sugar for a maximum fermentation by all the yeasts. Dextrose instead of sucrose was added to the cider in which the S and R forms of *Pichia alcoholophila* were to be grown, since this yeast ferments dextrose very slightly but does not ferment sucrose at all. After autoclaving, the cider used for fermentation by the S and R forms of *P. alcoholophila* had a pH value of 3.6, and 0.357 g per 100 cc of total acid calculated as malic acid. The cider used for fermentation by the two forms of *Saccharomyces cerevisiae* Saaz had an initial pH value of 3.6 and contained 0.400 g of acid per 100 cc calculated as malic. The cider used for fermentation by the two forms of *S. aceris-sacchari* and the two forms of *Willia anomala* had an initial pH value of 3.8 and contained 0.340 g of acid calculated as malic per 100 cc.

After the cider had been sterilized but before it was inoculated with the yeasts, an analysis was made to determine the presence of esters and alcohol. The results were negative. This procedure was followed with all the other media used, and with these also the results were negative.

The malt-extract broth consisted of 0.5 percent of sodium chloride, 0.3 percent of meat extract (Liebig's), 1.0 percent of peptone (Bacto), 10 percent of dextrose, and 2 percent of Trommer's malt extract.

[3] FABIAN, F. W., and MCCULLOUGH, N. B. DISSOCIATION IN YEASTS. Jour. Bact. 27: 583-622, illus. 1934.

Trommer's malt extract was used because it is high in total solids and produces excellent growth. All of the malt-extract broth needed was prepared at one time. After autoclaving it contained 0.139 g of total acids per 100 cc of medium calculated as lactic acid. The pH value was 5.2.

The synthetic medium contained 0.10 g each of monobasic potassium phosphate, monobasic ammonium phosphate, calcium sulphate, and magnesium sulphate, 10.0 g of sucrose, and 90 cc of distilled water. The reaction of the medium was adjusted with concentrated sulphuric acid. This medium also was made up in one batch. After autoclaving it had a pH value of 4.5 and contained 0.105 g of total acids per 100 cc of medium, calculated as sulphuric acid.

INOCULATION OF MEDIA

The media were placed in 6-liter Erlenmeyer flasks, each flask receiving 5 liters of the medium. These flasks were fitted with rubber stoppers through which a tube of glass and a siphon assembly passed. The tube of glass provided for the interchange of gases and was plugged with cotton. The siphon provided for the removal of samples of media. The latter had two Hoffman clamps separated by a glass tube, thus lessening the danger of contaminating the medium. After autoclaving, the rubber stoppers were given a heavy coating of paraffin.

The media in the flasks were incoculated with 2 percent of 24-hour cultures of the various yeast forms. Extra precautions against contamination were observed by placing the flasks inside a steamer which had been running just previously for several minutes.

CHEMICAL AND YEAST ANALYSIS

The methods of analysis used, unless otherwise stated, were those of the Association of Official Agricultural Chemists.[4] The analyses include pH, total acidity, volatile acids, nonvolatile acids, alcohol, and ester determinations. At the same time that the chemical analysis was made, the cultures were plated on malt-extract agar in order to check the cultures for contamination and to see whether any dissociation was taking place within the fermenting media, and in case there was, to determine the extent of the dissociation. The pH was determined by a quinhydrone electrode. Preparation of the sample was carried out according to the method of the Association of Official Agricultural Chemists, XVII, 23. The method used for determining the total acidity is that given under XVII, 44, except that 10 cc of the sample was diluted with 90 cc of distilled water before titrating in order to give a clearer end point. Factors of 0.0067, 0.0049, and 0.0090 were used in calculating the total acidity as malic, sulphuric, and lactic, respectively. The volatile acids were determined according to the procedure outlined under XVII, 45. The nonvolatile acids were determined according to the procedure given under XVII, 47, except that they were expressed as malic, sulphuric, and lactic acid and factors of 1.116, 0.817, and 1.500, respectively, were used. Alcohol was determined by neutralizing 100 cc of the sample with 20-percent sodium hydroxide solution, dis-

[4] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 3, 593 pp., illus. Washington, D. C. 1930.

tilling, and then obtaining the percentage by volume of alcohol by a Tag ebullioscope. The ester determination is essentially the procedure given under XVII, 65. However, it was necessary to use from 25 to 150 cc of excess alkali, depending upon the amount of esters in the sample. All analyses were run in duplicate.

TABLE 1.—*Analyses (percent) of cider, malt-extract broth, and a synthetic medium containing 10 percent of sucrose or dextrose, during fermentation by the S and R forms of Saccharomyces aceris-sacchari, Willia anomala, Pichia alcoholophila, and S. cerevisiae Saaz*

SYNTHETIC MEDIUM CONTAINING 10 PERCENT SUCROSE, S FORM OF S. ACERIS-SACCHARI

Number of days fermented	pH	Titratable acidity	Volatile acid as acetic	Nonvolatile acid	Alcohol by volume	Esters as ethyl acetate	Percentage of S and R or SR cells in medium
0	4.5	0.105	0.001	0.104	0.00	0.00	
15	3.3	.176	.037	.146	1.69	.03	100 S.
30	2.8	.251	.075	.190	3.20	.05	100 S.
45	2.6	.263	.128	.158	4.56	.10	100 S.
60	2.6	.317	.198	.155	4.90	.27	100 S.
75	2.6	.343	.226	.158	5.27	.51	100 S.
90	2.5	.361	.239	.166	5.30	.70	100 S.

SYNTHETIC MEDIUM CONTAINING 10 PERCENT SUCROSE, R FORM OF S. ACERIS-SACCHARI [1]

0	4.5	0.105	0.001	0.104	0.00	0.00	
15	2.7	.167	.029	.143	.88	.05	100 R.
30	2.6	.226	.089	.153	2.30	.11	100 R.
45	2.5	.275	.151	.152	2.38	.27	100 R.
60	2.5	.322	.198	.160	3.47	.43	100 R.
75	2.5	.359	.254	.153	1.80	.77	100 R.
90	2.5	.562	.407	.230	2.34	.60	100 R.

CIDER CONTAINING 10 PERCENT SUCROSE, S FORM OF S. ACERIS-SACCHARI [2]

0	3.8	0.340	0.008	0.331	0.00	0.00	
5	4.0	.351	.017	.332	.18	.01	100 S.
15	3.7	.469	.079	.381	1.81	----------	100 S.
25	3.7	.513	.129	.369	3.98	.03	100 S.
35	3.6	.521	.146	.358	4.87	.07	100 S.
45	3.8	.501	.158	.325	6.55	.18	100 S.
55	3.6	.481	.145	.320	6.66	.39	100 S.
65	3.7	.450	.097	.342	7.79	.68	100 S.
75	3.7	.381	.074	.298	7.50	.87	100 S.

CIDER CONTAINING 10 PERCENT SUCROSE, R FORM OF S. ACERIS-SACCHARI [2]

0	3.8	0.340	0.008	0.331	0.00	0.00	
5	3.8	.373	.017	.354	.26	.00	100 R.
15	3.7	.430	.042	.382	.81	.01	100 R.
25	3.7	.507	.088	.409	1.68	.04	100 R.
35	3.6	.555	.146	.392	2.71	.06	100 R.
45	3.7	.582	.207	.351	3.38	.10	100 R.
55	3.5	.612	.240	.344	3.82	.27	100 R.
65	3.5	.553	.194	.337	5.00	.55	100 R.
75	3.7	.480	.134	.330	4.95	.79	100 R.

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, S FORM OF S. ACERIS-SACCHARI [3]

0	5.2	0.139	0.002	0.136	0.00	0.00	
15	4.1	.383	.075	.271	3.70	.02	9 S, 91 SR.
30	3.8	.504	.128	.312	5.86	.10	7 S, 93 SR.
45	3.8	.601	.238	.244	5.60	.41	68 S, 42 SR.
60	4.1	.543	.224	.207	5.36	.96	20 S, 80 SR.
75	3.9	.371	.121	.190	5.20	1.37	63 S, 73 SR.

[1] See footnotes at end of table.

TABLE 1.—*Analyses (percent) of cider, malt-extract broth, and a synthetic medium containing 10 percent of sucrose or dextrose, during fermentation by the S and R forms of Saccharomyces aceris-sacchari, Willia anomala, Pichia alcoholophila, and S. cerevisiae Saaz*—Continued

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, R FORM OF S. ACERIS-SACCHARI [3]

Number of days fermented	pH	Titratable acidity	Volatile acid as acetic	Nonvolatile acid	Alcohol by volume	Esters as ethyl acetate	Percentage of S and R or SR cells in medium
0	5.2	0.139	0.002	0.136	0.00	0.00	
15	4.1	.318	.035	.266	1.12	.02	100 R.
30	3.7	.578	.088	.347	2.75	.04	98 R, 2 SR.
45	3.6	.794	.176	.530	4.16	.16	25 R, 75 SR.
60	3.7	.788	.214	.467	4.80	.42	3 R, 97 SR.
75	3.7	.715	.156	.481	4.65	.81	100 S.

SYNTHETIC MEDIUM CONTAINING 10 PERCENT SUCROSE, SR [4] FORM OF W. ANOMALA [1]

0	4.5	0.105	0.001	0.104	0.00	0.00	
15	2.8	.158	.022	.140	.50	.03	100 SR.
30	2.9	.212	.068	.157	1.12	.09	100 SR.
45	2.5	.278	.169	.140	1.33	.25	100 SR.
60	2.4	.364	.291	.126	1.19	.36	100 SR.
75	2.6	.337	.281	.107	1.40	.74	100 SR.
90	2.4	.428	.368	.127	.82	.67	100 SR.

SYNTHETIC MEDIUM CONTAINING 10 PERCENT SUCROSE, R FORM OF W. ANOMALA [1]

0	4.5	0.105	0.001	0.104	0.00	0.00	
15	2.8	.152	.025	.132	.49	.03	100 R.
30	2.9	.218	.083	.150	.76	.11	100 R.
45	2.5	.271	.178	.126	.98	.25	100 R.
60	2.3	.369	.288	.134	.76	.29	100 R.
75	2.4	.355	.263	.140	1.12	.46	100 R.
90	2.3	.443	----------	----------	.94	.42	100 R.

CIDER CONTAINING 10 PERCENT SUCROSE, SR [4] FORM OF W. ANOMALA [2]

0	3.8	0.340	0.008	0.331	0.00	0.00	
5	3.9	.362	.010	.351	.25	.00	100 SR.
15	3.6	.415	.027	.385	.95	.05	100 SR.
25	3.7	.443	.048	.389	1.57	.10	100 SR.
35	3.6	.476	.088	.378	2.08	.14	100 SR.
45	3.6	.485	.127	.343	1.26	.28	2 R, 80 SR, 18 S.
55	3.5	.499	.137	.346	2.22	.52	2 R, 60 SR, 38 S.
65	3.5	.493	.12	.355	1.80	.82	40 SR, 60 S.
75	3.7	.444	.170	.255	2.52	.96	7 SR, 93 S.

CIDER CONTAINING 10 PERCENT SUCROSE, R FORM OF W. ANOMALA [2]

0	3.8	0.340	0.008	0.331	0.00	0.00	
5	4.0	.363	.010	.355	.18	.00	100 R.
15	3.6	.423	.038	.381	.50	.04	100 R.
25	3.7	.452	.075	.368	.81	.09	100 R.
35	3.6	.510	.107	.391	1.00	.18	100 R.
45	3.5	.515	.142	.357	1.29	.25	100 R.
55	3.5	.561	.185	.355	1.10	.40	100 R.
65	3.5	.545	.184	.390	----------	.47	100 R.
75	3.7	.444	.125	.305	1.22	.74	100 R.

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, SR [4] FORM OF W. ANOMALA [3]

0	5.2	0.139	0.002	0.136	0.00	0.00	
15	3.5	.468	.040	.408	1.61	.03	100 SR.
30	3.5	.602	.076	.488	3.92	.10	2 S, 95 SR, 3 R.
45	3.5	.681	----------	----------	5.82	.21	29 S, 71 SR.
60	3.7	.674	.174	.413	6.35	.49	73 S, 27 SR.
75	3.6	.715	.174	.444	5.45	.88	60 R, 40 SR.

See footnotes at end of table.

TABLE 1.—*Analyses (percent) of cider, malt-extract broth, and a synthetic medium, containing 10 percent of sucrose or dextrose, during fermentation by the S and R forms of Saccharomyces aceris-sacchari, Willia anomala, Pichia alcoholophila, and S. cerevisiae Saaz*—Continued

MALT-EXTRACT BROTH CONTAINING 10 PERCENT SUCROSE, R FORM OF W. ANOMALA [3]

Number of days fermented	pH	Titratable acidity	Volatile acid as acetic	Nonvolatile acid	Alcohol by volume	Esters as ethyl acetate	Percentage of S and R or SR cells in medium
0	5.2	0.139	0.002	0.136	0.00	0.00	
15	4.3	.249	.028	.207	1.04	.05	100 R.
30	3.5	.567	.070	.464	1.97	.06	100 R.
45	3.5	.668	.163	.424	2.50	.23	100 R.
60	3.7	.725	.186	.446	3.02	.96	96 R, 4 SR.
75	3.6	.615	.153	.386	1.35	1.13	99 R, 1 S.

CIDER CONTAINING 10 PERCENT DEXTROSE, S FORM OF P. ALCOHOLOPHILA [3]

0	3.6	0.357	0.006	0.351	0.00	0.00	
5	3.9	.362	.008	.354	.11	.00	100 S.
15	4.1	.397	.009	.338	.09	.00	98 S, 2 R.
25	3.8	.344	.012	.331	.12	.00	76 S, 24 R.
35	3.6	.355	.015	.337	.13	.00	26 S, 74 R.
45	3.7	.338	.017	.319	.18	.00	12 S, 88 R.
55	3.9	.290	.014	.279	.18	.00	10 S, 90 R.
65	3.7	.239	.025	.211	.12	.00	7 S, 94 R.
75	3.9	.186	.018	.165	.48	.00	4 S, 96 R.

CIDER CONTAINING 10 PERCENT DEXTROSE, R FORM OF P. ALCOHOLOPHILA [3]

0	3.6	0.357	0.006	0.351	0.00	0.00	
5	3.8	.354	.009	.344	.10	.00	100 R.
15	3.9	.351	.008	.342	.08	.00	100 R.
25	3.9	.348	.013	.333	.07	.00	100 R.
35	3.6	.354	.012	.342	.12	.00	100 R.
45	3.7	.337	.014	.322	.10	.00	100 R.
55	3.9	.301	.016	.283	.18	.00	100 R.
65	3.7	.248	.017	.229	.31	.00	100 R.
75	3.9	.217	.019	.195	.40	.00	100 R.

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, S FORM OF P. ALCOHOLOPHILA [3]

0	5.2	0.139	0.002	0.139	0.00	0.00	
15	5.1	.141	.003	.136	.20	.00	100 S.
30	4.9	.191	.009	.178	.14	.00	95 SR, 5 S.
45	4.8	.249	.012	.231	.25	.01	72 R, 14 SR, 14 S.
60	4.5	.230	.008	.219	.22	.01	100 R.
75	4.2	.256	.007	.245	.20	.02	89 SR, 7 R, 4 S.

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, R FORM OF P. ALCOHOLOPHILA [3]

0	5.2	0.139	0.002	0.136	0.00	0.00	100 R.
15	5.1	.149	.002	.115	.13	.00	100 R.
30	4.9	.207	----------	----------	.25	.00	100 R.
45	4.8	.246	.026	.207	.21	.01	100 R.
60	4.5	.255	.014	.234	.20	.01	100 R.
75	4.0	.288	.013	.269	.25	.00	100 R.

CIDER CONTAINING 10 PERCENT SUCROSE, S FORM OF S. CEREVISIAE SAAZ [3]

0	3.6	0.400	0.006	0.393	0.00	0.00	
5	3.8	.480	.009	.470	.25	.00	100 S.
15	3.6	.547	.046	.996	1.94	----------	100 S.
25	3.6	.496	.073	.415	4.25	.01	100 S.
35	3.6	.515	.070	.437	4.42	.01	100 S.
45	3.6	.550	.080	.461	5.05	.01	100 S.
55	3.5	.493	.077	.407	5.55	.01	[5].
65	3.5	.484	.081	.395	5.87	.00	[6].
75	3.3	.480	.069	.402	5.41	.00	[6].

See footnotes at end of table.

TABLE 1—*Analyses (percent) of cider, malt-extract broth, and a synthetic medium containing 10 percent of sucrose or dextrose, during fermentation by the S and R forms of Saccharomyces, aceris-sacchari, Willia anomala, Picha alcoholophila, and S. cerevisiae Saaz*—Continued.

CIDER CONTAINING 10 PERCENT SUCROSE, R FORM OF S. CEREVISIAE SAAZ [2]

Number of days fermented	pH	Titratable acidity	Volatile acid as acetic	Nonvolatile acid	Alcohol by volume	Esters as ethyl acetate	Percentage of S and R or SR cells in medium
0	3.6	0.400	0.006	0.393	0.00	0.00	
5	3.9	.473	.012	.460	.31	.00	100 R.
15	3.5	.488	.029	.456	.77	.03	100 R.
25	3.6	.492	.047	.440	1.25	.07	100 R.
35	3.6	.524	.070	.446	1.28	.14	100 R.
45	3.5	.579	.112	.454	1.46	.22	100 R.
55	3.3	.591	.128	.448	1.90	.36	100 R.
65	3.3	.521	.105	.404	1.21	.52	100 R.
75	3.1	.479	.064	.408	.78	1.00	100 R.

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, S FORM OF S. CEREVISIAE SAAZ [3]

0	5.2	0.139	0.002	0.136	0.00	0.00	
15	4.3	.292	.047	.222	4.66	.00	100 S.
30	4.3	.341	.054	.260	6.25	.00	100 S.
45	4.3	.309	.054	.228	6.72	.01	[5].
60	4.3	.295	.060	.205	6.26	.01	95 S, 5 R.[7]
75	4.1	.296	.059	.207	6.72	.02	74 SR, 26 S.[8]

MALT-EXTRACT BROTH CONTAINING 10 PERCENT DEXTROSE, R FORM OF S. CEREVISIAE SAAZ [3]

0	5.2	0.139	0.002	0.136	0.00	0.00	100 R.
15	4.4	.249	.030	.204	1.48	.02	100 R.
30	4.1	.374	.072	.266	3.00	.09	100 R.
45	4.0	.476	.067	.217	4.35	.27	100 R.
60	3.8	.585	.193	.296	4.51	.72	100 R.
75	3.9	.504	.162	.261	5.18	1.07	100 R.

[1] Titratable acidity and nonvolatile acid as sulphuric.
[2] Titratable acidity and nonvolatile acid as malic.
[3] Titratable acidity and nonvolatile acid as lactic.
[4] Some revision of S to R Form.
[5] No growth in 1 loopful.
[6] No growth in 5 cc.
[7] Scant growth in 5 cc.
[8] Moderate growth in 5 cc.

RESULTS OF EXPERIMENTS

ANALYSES

The data given in table 1 show that in the cider and in the malt-extract broth the S form of *Saccharomyces aceris-sacchari* reached a peak in volatile acid production by the forty-fifth day. This peak was followed by a rapid decline in the concentration of the volatile acids until the seventy-fifth day, when approximately 50 percent of the maximum amount remained. In the synthetic medium (table 1) the volatile acids increased steadily up to the ninetieth day, however, the concentration at that time equaling the concentration of the volatile acids in the malt-extract broth when at their peak. It is interesting to note that, although the maximum production of volatile acids in the cider reached little more than half the maximum production in either of the other two media, a decline in concentration of the volatile acids nevertheless took place. Table 1 shows that by the seventy-fifth day the percentage of alcohol was practically the same

in the synthetic medium and in malt-extract broth, but that the cider contained between 40 and 50 percent more alcohol. Ester production was 24 percent greater in the cider than in the synthetic medium, and 96 percent greater in the malt-extract broth than in the synthetic medium.

During the 75-day fermentation period dissociation occurred in the yeasts. However, the increase in ester production is directly attributable to the action of the S forms on the malt-extract medium rather than to the intermediate forms of yeasts, since, as will be shown later, the R form of this yeast produced less esters than did the S form.

Figure 1, *A*, shows the relationship between alcohol, volatile acids, and esters produced by the S form of *Saccharomyces aceris-sacchari* in

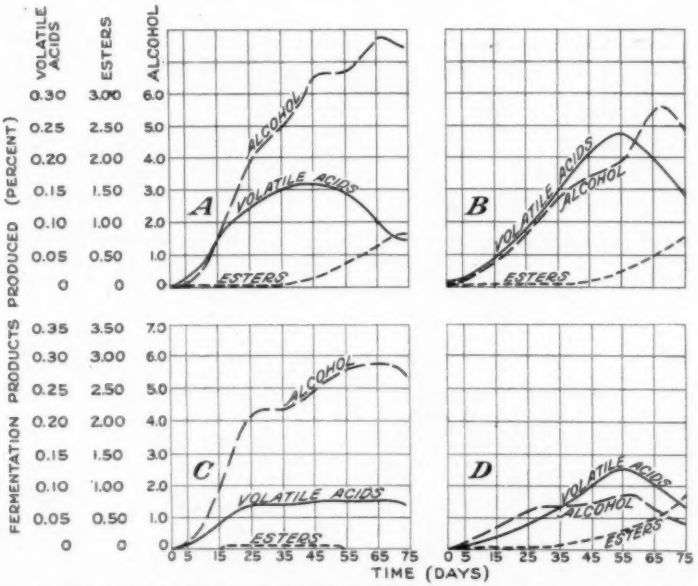

FIGURE 1.—Relationship between the alcohol, volatile acids, and esters produced by the S and R forms of *Saccharomyces aceris-sacchari* and *S. cerevisiae* Saaz in cider; the ratios used in plotting alcohol, esters, and acids being 1:2:20, respectively; *A*, S form of *S. aceris-sacchari*; *B*, R form of *S. aceris-sacchari*; *C*, S form of *S. cerevisiae* Saaz; *D*, R form of *S. cerevisiae* Saaz.

cider. It will be noted that the amount of volatile acids and alcohol increased rapidly after the fifth day, though the ester production was very scant until approximately the fortieth day, after which it increased rapidly.

Table 1 shows that the volatile acids produced by the R form of *Saccharomyces aceris-sacchari*, as well as by the S form, increased continuously in the synthetic medium throughout the experiment, but decreased after 50 or more days in cider and malt-extract broth. Unlike the condition obtaining with the S form, the concentration of volatile acids produced by the R form in the synthetic medium at the seventy-fifth day was about 6 percent greater than the greatest concentration of this product in either cider or malt-extract broth. At 60 days the alcohol production had reached its peak in all three media.

The cider (shown at 65 days) showed a peak alcohol concentration of 5.60 percent, malt-extract broth 4.80 percent, and synthetic medium, 3.47 percent by volume. The ester production in all three media was about the same. Figure 1, *B*, shows graphically the production of fermentation products by the R form in cider. It will be noted that about 15 days after the production of esters started there was a rapid decrease in the volatile acids and later a decrease in the alcohol.

The amount of volatile acids produced by the SR form of *Willia anomala* (table 1), was approximately equal in the cider and in the malt-extract broth at the seventy-fifth day, but in the synthetic medium the amount produced was 61 and 65 percent more than in the cider and malt-extract broth, respectively. Alcohol production varied greatly in the different media. At the seventy-fifth day the amount of alcohol present in the synthetic medium was 1.40 percent, in cider 2.52 percent, and in the malt-extract broth 5.45 percent. The relatively large amount of alcohol in the last-mentioned medium may have been due in part to the presence of a large percentage of true S cells which appeared in the medium during the latter part of the fermentation. The difference in ester production was not large. The cider had the highest concentration of esters and the synthetic medium the lowest.

The R form of *Willia anomala* reached a peak of 0.174 and 0.186 g of volatile acids per 100 cc in malt-extract broth and cider, respectively, at the sixtieth day (table 1). In synthetic medium a peak of 0.288 g per 100 cc was reached at the same time. In the malt-extract broth approximately 135 percent more alcohol was produced than in the cider and 170 percent more than in the synthetic medium. These differences can only be due to the composition of the media, as practically no dissociation took place in any of the three media. The amount of ester production on the seventy-fifth day was 0.46, 0.74, and 1.13 g per 100 cc in the synthetic medium, cider, and malt-extract broth, respectively.

None of the S and R forms of *Pichia alcoholophila* or *Saccharomyces cerevisiae* Saaz grew in the synthetic medium. Therefore, only the data concerning the fermentation of these yeasts in cider and malt-extract broth are presented.

Table 1 indicates the slight fermentation produced by the S form of *Pichia alcoholophila*. In malt-extract broth the greatest concentration of volatile acids was 0.012 g per 100 cc, while in cider it was 0.025 g per 100 cc. The alcohol concentration in both media varied considerably during the fermentation, but did not rise above 0.48 percent. Only traces of esters were produced.

The fermentation produced by the R form of *Pichia alcoholophila* (table 1) was nearly the same as the fermentation produced by the S form, but more volatile acids were produced in cider than in the malt-extract broth. The slight fermentations produced by the S and R forms were not due to poor growth of the yeast forms, since both grew abundantly in the two media used.

Table 1 shows that the maximum amount of volatile acids produced by the S form of *Saccharomyces cerevisiae* Saaz in the cider exceeded that produced in malt-extract broth by 35 percent. The alcohol production was much more rapid in malt-extract broth and reached a higher maximum than in cider. Only a very small amount of esters was produced, the highest concentration being 0.02 g per 100 cc in

malt-extract broth. When one loopful of malt-extract broth was plated on the forty-fifth day, no colonies resulted. At 60 days 5 cc of this medium produced only a few colonies when plated. At the seventy-fifth day 5 cc of the culture produced about 400 colonies. The culture was dead in cider by the fifty-fifth day. Plates inoculated with 5 cc of the medium produced no colonies. Figure 1, *C*, shows the course of fermentation by the S form in cider.

Table 1 shows that the R form of *Saccharomyces cererisiae* Saaz produced 50 percent more volatile acids and 173 percent more alcohol in malt-extract broth than in cider. The ester production was nearly the same in the two media, about 1 g per 100 cc. Figure 1, *D*, shows a rapid decrease in alcohols and volatile acids after the fifty-seventh day. The esters, however, continued the rapid rise which started about the twenty-fifth day.

The last column in table 1 shows the amount of dissociation which took place in terms of the percentage of cells that had reverted at the end of 60 days. In no case was there any change of form in the synthetic medium. Four of the forms which dissociated in malt-extract broth did not dissociate in cider. Furthermore, the malt-extract broth contained a greater number of dissociated forms at 60 days than did the cider in which the same species and form of yeast was growing.

INFLUENCE OF OXYGEN ON ESTER PRODUCTION

In order to study the influence of oxygen on ester production an experiment was set up in which the amount of air in contact with the fermenting solution varied considerably. For this experiment the R form of *Saccharomyces cerevisiae* Saaz was seeded in malt-extract broth containing 10 percent of dextrose and 2 percent of malt extract. Previous experiments had demonstrated that this combination of yeast and medium would produce a high ester content. A 6-liter flask containing 5 liters of the medium was seeded with the yeast. The only access of air was through a hole 15 mm in diameter which was tightly stoppered with cotton. The flask was allowed to remain undisturbed during the fermentation. At the end of 10 days less than 0.02 g per 100 cc of esters had formed. Five liters of medium was placed in a second 6-liter flask and the 50-mm opening to the flask plugged with cotton. After inoculation and every other day thereafter the flask was shaken in order to incorporate air into the medium. At the end of 10 days 0.58 g of esters per 100 cc had been produced.

A third 5 liters of medium was placed in a sterile pan 16 inches square. The medium covered the pan to a depth of about 1 inch. To prevent contamination the pan was covered with several layers of sterile cheesecloth. The contents were not agitated during the fermentation. The yeast made an extremely abundant growth, but at the end of 10 days there were no esters present.

This experiment indicates that the conditions under which yeasts are grown influence the amount of esters produced. When oxygen is low, as in the first flask, ester production is low. When oxygen is abundant, as in the pan, no esters are formed. The ideal condition, or at least the most favorable condition of the three tried here, was when the amount of oxygen present was sufficient to maintain a normal growth, as in the second flask.

DISCUSSION

The comparison of the products of fermentation of the S and R forms of *Saccharomyces aceris-sacchari* as given in table 1, shows that the medium is a more important factor than the form of yeast in determining the amount of volatile acids produced. To illustrate, the R form produced much more volatile acids in the synthetic medium and in cider than the S form did, but the S form produced slightly more in the malt-extract broth than did the R form. Without exception the S form produced more alcohol than did the R form in the same kinds of media. In cider and malt-extract media the S form produced more esters than the R form; the reverse was true in the synthetic medium.

Table 1 shows no significant difference in the production of volatile acids in the three media between the SR and R forms of *Willia anomala*. In all media the SR form produced more alcohol than the R form. The difference was not great in the synthetic medium, but in malt-extract broth the SR form produced 110 percent more alcohol than did the R form. Ester production varied with the kind of medium used. In malt-extract broth the R form produced 28 percent more esters than did the SR form, but the SR form produced 61 percent more in the synthetic medium and 30 percent more in the cider.

Table 1 indicates that there was no significant difference between the products of fermentation of the S and R forms of *Pichia alcoholophila*.

The R form of *Saccharomyces cerevisiae* Saaz (table 1) produced 221 percent more volatile acids in malt-extract broth and 58 percent more in cider than did the S form. The S form far exceeded the R form in alcohol production in both media, the ratio in cider being approximately 3:1 and in malt-extract broth 1.3:1.0. The S form produced practically no esters, but the R form produced approximately 1 g per 100 cc in both media.

The S and SR forms of the four yeasts vary to a marked degree in their fermentations. Table 1 shows that the ratio of the maximum production of volatile acids in malt-extract broth by the S or SR forms of *Saccharomyces aceris-sacchari, Willia anomala, S. cerevisiae* Saaz, and *Pichia alcoholophila* is 20:15:5:1, respectively.

The ratio for maximum alcohol production in the same order is 23:25:27:1, and the maximum ester ratio is 68:44:1:1. Figure 1, *B*, shows the fermentation of cider produced by the R form of *Saccharomyces aceris-sacchari*, which is a strong ester producer. It will be noted that about 15 days after the formation of esters began, the volatile acid concentration dropped rapidly. Figure 1, *C*, shows the fermentation of the same medium by the S form of *Saccharomyces cerevisiae* Saaz, which is a very weak ester producer. The volatile acids after 25 days of fermentation reached a rather constant concentration which was maintained throughout the rest of the fermentation. The drop in concentration of the volatile acids in the case of the S form of *S. aceris-sacchari* may have been due to the combination of volatile acids and alcohol to form esters. The drop in the alcohol concentration would not be as noticeable as the drop in volatile acids concentration because of the comparatively large amount of the former in the media. *B* and *D*, of figure 1, which are graphs of fermentations caused by ester-producing yeast forms,

show the drop in volatile acid and alcohol which followed the rapid increase in ester production.

Table 1 shows that the R forms differ as much among themselves as do the S forms. The ratio of the maximum production of volatile acids in malt-extract broth by the R forms of *Saccharomyces aceris-sacchari*, *Willia anomala*, *S. cerevisiae* Saaz, and *Pichia alcoholophila*, in the order named, is 8:7:7:1; of alcohol, 19:12:21:1; and of esters, 81:113:107:1.

SUMMARY

Detailed analyses of the products of fermentation of the S and R forms of the four yeasts studied, *Saccharomyces cerevisiae* Saaz, *S. aceris-sacchari*, *Pichia alcoholophila*, and *Willia anomala* in three different media, cider, malt-extract broth, and a synthetic medium in general show:

(1) In some species of yeasts there is a marked difference in the fermentation properties of the S and R forms; in other species there is little or no difference.

(2) The S forms produced more alcohol in a shorter length of time than did the R forms.

(3) The production of volatile acids was extremely variable. The amount produced varied with the different media, with the species of yeast, and with the S and R forms of the different species.

(4) The production of esters by the S and R forms of the four species of yeasts does not show as great variation as the productions of volatile acid. There are some exceptions to this. The most important is the S form of *S. cerevisiae* Saaz in malt-extract broth and cider. In these media the amount of esters produced by the S form is practically nil while the amount produced by the R form is 1 g per 100 cc. Another exception is in malt-extract broth where the S form of *S. aceris-sacchari* produced 69 percent more esters than the R form. The SR form of *W. anomala* produced 30 percent more esters in cider than the R form and 61 percent more in the synthetic medium. *P. alcoholophila* produced practically no esters in any of the media.

(5) The conditions under which the yeasts are grown influence the ester production. Sufficient oxygen to maintain normal growth produced the largest amount of esters. When there was an abundance of oxygen or insufficient oxygen no esters were produced.

(6) In the yeasts which produced esters, the esters did not appear in appreciable quantities until 35 to 45 days after inoculation. Shortly after the esters appeared there was a corresponding decrease in alcohol and volatile acids. In the case of the S form of *S. cerevisiae* Saaz there was a moderate volatile acid and high alcoholic content in malt-extract broth, but practically no esters were formed.

(7) The three media used affected the stability of the yeasts differently. The S and R forms of the species which grew in the synthetic medium were very stable in that medium, the S form showed no tendency to change to the R form, nor the R form to change to the S form. In the cider, however, the tendency to change was greater and in the malt-extract broth it was still greater.

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